82936

STIC-Biotech/ChemLib

From: STIC-ILL
Sent: Monday, December 23, 2002 6:48 AM
To: STIC-Biotech/ChemLib
Subject: RE: RUSH sequence search for 09/825,580

09/825,580

-----Original Message-----
From: Gambel, Phillip
Sent: Sunday, December 22, 2002 10:06 AM
T : STIC-ILL
Cc: Chan, Christina
Subject: RUSH sequence search for 09/825,580

stic

please provide a sequence and a sequence interference search for

ussn 09 / 825,580

SEQ ID NO: 2

thanx

phillip gambel
art unit 1644
308-3997

1644 mailbox 9E12

---

christina

please okay a rush sequence search for this case

thanx

Point of Contact:
Toby Port
Technical Info. Specialist
CM1 6A04
703-308-3534

	TYPE OF SEARCH:	VENDOR/COST (where applic.)
Searcher:_____	NA Sequences:_____	STN:_____
Phone:_____	AA Sequences:_____	DIALOG:_____
Location:_____	Structures:_____	Questel/Orbit:_____
Date Picked Up: 12/23	Bibliographic:_____	DRLink:_____
Date Completed: 12/23	Litigation:_____	Lexis/Nexis:_____
Searcher Prep/Review:_____	Full text:_____	Sequence Sys.:_____
Clerical:_____	Patent Family:_____	WWW/Internet:_____
Online time:_____	Other:_____	Other (specify):_____

STIC-ILL

NPL

From: Gambel, Phillip
Sent: Monday, December 23, 2002 8:27 AM
To: STIC-ILL
Subject: ltc4 and p-selectin

stic

please provide the following references to

phillip gambel
art unit 1644
308-3997

1644 mailbox 9E12

3/7/12 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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122029779 CA: 122(3)29779j JOURNAL
Polymorphonuclear leukocyte-platelet interaction: role of P-selectin in thromboxane B2 and leukotriene C4 cooperative synthesis
AUTHOR(S): Maugeri, Norma; Evangelista, Virgillio; Celardo, Antonio; Dell'Eba, Giuseppe; Martelli, Nicola; Piccardoni, Paola; de Gaetano, Giovanni; Cerletti, Chiara
LOCATION: Giulio Bizzozero Lab. Platelet Leukocyte Pharmacol., Ist. Ricerche Farmacol. Mario Negri, Santa Maria Imbaro, Italy
JOURNAL: Thromb. Haemostasis DATE: 1994 VOLUME: 72 NUMBER: 3 PAGES: 450-6 CODEN: THHADQ ISSN: 0340-6245 LANGUAGE: English
SECTION:
CA215010 Immunochemistry
CA202XXX Mammalian Hormones
IDENTIFIERS: polymorphonuclear leukocyte platelet adhesion P selectin, TXB2 LTC4 P selectin leukocyte platelet
DESCRIPTORS:
Adhesion,bio-... Blood platelet... Leukocyte,polymorphonuclear... Receptors,P-selectins...
role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.
CAS REGISTRY NUMBERS:
506-32-1 arachidonic acid metab. modulated by cathepsin G-induced expression of P-selectin on platelet surface
56645-49-9 polymorphonuclear leukocyte-derived cathepsin G induces the expression of P-selectin on platelet surface
54397-85-2 72025-60-6 role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.

3/7/11 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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123283576 CA: 123(21)283576m JOURNAL
Leukotriene C4/D4 induces P-selectin and sialyl Lewisx-dependent alterations in leukocyte kinetics in vivo
AUTHOR(S): Kanwar, Samina; Johnston, Brent; Kubes, Paul
LOCATION: Immunology Research Group, Univ. of Calgary, Calgary, Can.,
JOURNAL: Circ. Res. DATE: 1995 VOLUME: 77 NUMBER: 5 PAGES: 879-87
CODEN: CIRUAL ISSN: 0009-7330 LANGUAGE: English
SECTION:

3/7/4 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09587802 98026174 PMID: 9379052
Differential roles of selectins and the alpha4-integrin in acute, subacute, and chronic leukocyte recruitment in vivo.
Johnston B; Walter U M; Issekutz A C; Issekutz T B; Anderson D C; Kubes P
Immunology Research Group, University of Calgary, Alberta, Canada.
Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Nov 1 1997, 159 (9) p4514-23, ISSN 0022-1767 Journal Code: 2985117R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adhesion blocking mAbs specific for rat P-, E-, and L-selectin and the alpha4-integrin were used to characterize leukocyte recruitment mechanisms in models of LTC4 (acute), LPS (subacute), and adjuvant-induced (chronic) inflammation. Intravital microscopy was employed to measure leukocyte rolling and adhesion in rat mesenteric venules. Superfusing the mesentery with 20 nM LTC4 elicited an increase in leukocyte rolling (66.8 +/- 3.8 vs 18.2 +/- 3.2 cells/min control) that was completely eliminated by an anti-rat P-selectin mAb. Superfusion with 1 microg/ml LPS induced a significant increase in leukocyte rolling within 15 min (73 +/- 8 vs 33 +/- 6 cells/min control). Rolling increased further starting at 105 min and peaked by 150 min (141 +/- 23 cells/min). LPS-induced leukocyte rolling was eliminated during the first 90 min by the P-selectin mAb. The later increase in leukocyte rolling was not prevented by a second treatment with P-selectin mAb or a function-blocking mAb against rat E-selectin. This later phase of leukocyte rolling was blocked by treatments with mAbs against either the alpha4-integrin or L-selectin. Twelve days following Mycobacterium butyricum immunization, 300 to 500 rolling cells/min were observed. This could be reduced approximately 50 to 60% by mAb against either the alpha4-integrin or L-selectin. The combination of both mAbs eliminated approximately 90% of rolling. Neither the P- nor E-selectin mAbs reduced rolling in this chronic inflammatory model. This study highlights differences in leukocyte adhesive mechanisms elicited by different stimuli and at different time points within the same vascular bed.
Record Date Created: 19971112

3/7/2 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11163195 21190512 PMID: 11296849
Soluble P-selectin antagonist mediates rolling velocity and adhesion of leukocytes in acutely inflamed venules.
Eppihimer M J; Schaub R G
Wyeth/Genetics Institute, Andover, MA 01810, USA. meppihimer@genetics.com
Microcirculation (New York, N.Y. : 1994) (United States) Feb 2001, 8 (1) p15-24, ISSN 1073-9688 Journal Code: 9434935
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
OBJECTIVE: Leukocyte rolling is recognized as an important event in facilitating the extravasation of leukocytes from the vascular to the interstitial compartment, and is mediated by the selectin family of cell adhesion molecules. The aim of this study was to evaluate and characterize the rolling behavior of leukocytes in a model of acute inflammation using a novel soluble selectin ligand directed against P-selectin.
METHODS: Feline mesenteric postcapillary venules were visualized using intravital microscopy prior to and following exposure to leukotriene C4 (LTC4) in animals pretreated with vehicle (saline) and the P-selectin antagonist rPSGL-Ig. RESULTS: A concentration of 500 pM LTC4 induced a threefold and sixfold elevation in leukocyte rolling flux and adhesion, respectively, compared to baseline values ($p < 0.05$). Administration of rPSGL-Ig had no effect on LTC4-induced leukocyte rolling flux but significantly attenuated the increase in the fraction of

Gambel, Phillip

From: igriffith@shaw.ca
Sent: Monday, December 23, 2002 11:33 AM
To: Phillip.Gambel@USPTO.GOV
Subject: Re: RE: FW: 2003 calendar...

apartment: (780) 989-0403
cell: (780) 717-3057

----- Original Message -----
From: Phillip.Gambel@USPTO.GOV
Date: Monday, December 23, 2002 9:31 am
Subject: RE: FW: 2003 calendar...

> could you send me your phone numbers
>
> i am almost positive i have them at home, but just in case they got
> misplaced
>
> i'll try to give you a call between now and new year's to see how
> you'redoing
>
>
>
> -----Original Message-----
> From: igriffith@shaw.ca [mailto:igriffith@shaw.ca]
> Sent: Monday, December 23, 2002 11:26 AM
> To: Phillip.Gambel@USPTO.GOV
> Subject: Re: FW: 2003 calendar...
>
>
> My, oh, my. Each invention speaks for itself.....
>
> ----- Original Message -----
> From: Phillip.Gambel@USPTO.GOV
> Date: Sunday, December 22, 2002 7:50 am
> Subject: FW: 2003 calendar...
>
> > enjoy
> >
> > phillip
> > Subject: FW: 2003 calendar...
> >
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> >
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> >
> >
> >
> >
> > You can print off a copy of this "Bizarre Patents 2003"
> calendar.
> > Or scroll
> > down to particular months. Definitely some good ones contained
> > within.
> > <
> >
> http://www.library.ubc.ca/patscan/calendar/Patscan%202003%20Calendar.pdf<"target="l">
http://www.library.ubc.ca/patscan/calendar/Patscan%202003%20Calen
> dar.pdf> >
> >
> >
>

Gambel, Phillip

From: Pronk, Jake (Chugach)
Sent: Monday, December 23, 2002 9:59 AM
To: Gambel, Phillip
Subject: Happy Holidays

I will be out of the office until January 6, 2003.

If you need assistance please contact:
Njeri High: (308-2234)
Kimberly Kenney: (305-2280)
Bill Thomas : (605-0481)
Felice Bray: (308-0905)

All other requests and e-mails I will answer upon my return.

Thank you and Happy Holidays!

Jake Pronk
Office Manager 1610/1620
Crystal Mall 1, 6B14
(703) 305-5470

425,208 11.1012

STIC-ILL

From: Gambel, Phillip
Sent: Monday, December 23, 2002 8:27 AM
To: STIC-ILL
Subject: ltc4 and p-selectin

stic

please provide the following references to

phillip gambel
art unit 1644
308-3997

1644 mailbox 9E12

9129300

3/7/12 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

122029779 CA: 122(3)29779j JOURNAL
Polymorphonuclear leukocyte-platelet interaction: role of P-selectin in thromboxane B2 and leukotriene C4 cooperative synthesis
AUTHOR(S): Maugeri, Norma; Evangelista, Virgillio; Celardo, Antonio; Dell'Eba, Giuseppe; Martelli, Nicola; Piccardoni, Paola; de Gaetano, Giovanni; Cerletti, Chiara
LOCATION: Giulio Bizzozero Lab. Platelet Leukocyte Pharmacol., Ist. Ricerche Farmacol. Mario Negri, Santa Maria Imbaro, Italy
JOURNAL: Thromb. Haemostasis DATE: 1994 VOLUME: 72 NUMBER: 3 PAGES: 450-6 CODEN: THHADQ ISSN: 0340-6245 LANGUAGE: English
SECTION:
CA215010 Immunochemistry
CA202XXX Mammalian Hormones
IDENTIFIERS: polymorphonuclear leukocyte platelet adhesion P selectin, TXB2 LTC4 P selectin leukocyte platelet
DESCRIPTORS:
Adhesion,bio-... Blood platelet... Leukocyte,polymorphonuclear... Receptors,P-selectins...
role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.
CAS REGISTRY NUMBERS:
506-32-1 arachidonic acid metab. modulated by cathepsin G-induced expression of P-selectin on platelet surface
56645-49-9 polymorphonuclear leukocyte-derived cathepsin G induces the expression of P-selectin on platelet surface
54397-85-2 72025-60-6 role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.

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3/7/11 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

123283576 CA: 123(21)283576m JOURNAL
Leukotriene C4/D4 induces P-selectin and sialyl Lewisx-dependent alterations in leukocyte kinetics in vivo
AUTHOR(S): Kanwar, Samina; Johnston, Brent; Kubes, Paul
LOCATION: Immunology Research Group, Univ. of Calgary, Calgary, Can.,
JOURNAL: Circ. Res. DATE: 1995 VOLUME: 77 NUMBER: 5 PAGES: 879-87
CODEN: CIRUAL ISSN: 0009-7330 LANGUAGE: English
SECTION:

Leukotriene C_4/D_4 Induces P-Selectin and Sialyl Lewis^x–Dependent Alterations in Leukocyte Kinetics In Vivo

Samina Kanwar, Brent Johnston, Paul Kubes

Abstract The objective of this study was to assess the effect of leukotriene C_4 (LTC_4) on the flux of rolling leukocytes, leukocyte rolling velocity, and leukocyte adhesion in postcapillary venules in vivo and to study the underlying molecular mechanisms involved. LTC_4 (20 nmol/L) induced a rapid and significant increase in leukocyte rolling flux that was inhibitable by an anti–P-selectin antibody and soluble sialyl Lewis^x (sLe^x). LTC_4 also induced a significant reduction in leukocyte rolling velocity, an event that was independent of P-selectin but entirely dependent on sLe^x. This LTC_4-induced reduction in leukocyte rolling velocity was independent of any hemodynamic alterations. Another P-selectin effector, histamine, did not affect leukocyte rolling velocity even at >5000 times the concentration of LTC_4. Treatment with an anti–L-selectin antibody had no effect on the LTC_4-induced increase in leukocyte rolling or reduction in rolling velocity. Inhibition of LTC_4 bioconversion to LTD_4 by pretreatment with L-serine (100 μmol/L) prevented the LTC_4-induced increase in leukocyte rolling flux and the LTC_4-induced reduction in leukocyte rolling velocity. A subtle, yet significant, increase in leukocyte adhesion was also observed with LTC_4. Pretreatment with a platelet-activating factor receptor antagonist returned the LTC_4-induced leukocyte rolling velocity to baseline levels. The addition of a very low concentration of platelet-activating factor (1 nmol/L) induced significant leukocyte adhesion in the presence of LTC_4 but not histamine. This study demonstrates that LTC_4, via bioconversion to leukotriene D_4, induces a P-selectin–dependent and sLe^x-dependent increase in leukocyte rolling flux and a P-selectin–independent but sLe^x-dependent reduction in leukocyte rolling velocity, a parameter that may play an essential role in subsequent leukocyte adhesion. (*Circ Res.* 1995;77:879-887.)

Key Words • leukotriene C_4 • leukocyte rolling • sialyl Lewis^x • P-selectin

Leukotriene C_4 (LTC_4), a cysteinyl leukotriene, is a biologically active lipid derived from the lipoxygenase-catalyzed metabolism of arachidonic acid.[1] It is a potent proinflammatory agent and has been implicated in a wide range of inflammatory conditions in a variety of organs.[1-5] Increased levels of LTC_4 are found in plasma, pulmonary edema fluid,[6,7] and bronchoalveolar and nasal lavage fluid in patients suffering from asthma,[8] allergic rhinitis,[9] cystic fibrosis,[10] and adult respiratory distress syndrome.[11] LTC_4 and its metabolic products have also been reported in the synovial fluid of patients with rheumatoid arthritis,[12] the small intestinal mucosa of children with celiac disease,[13] and psoriatic skin lesions.[14] In an experimental model, Rainsford[15] has recently reported increased LTC_4 levels in the efferent gastric circulation of pigs after indomethacin administration and postulated a role for LTC_4 in nonsteroidal anti-inflammatory drug–induced gastric mucosal injury. Finally, the concept that LTC_4 is involved in pathogenesis is further supported by the fact that LTC_4 infusion mimics the sequelae of inflammation. For example, in the gastrointestinal tract, LTC_4 induces mild gastric mucosal lesions and greatly augments gastric ulcer formation and intestinal mucosal damage in response to irritants including ethanol[16] and acidified acetylsalicylic acid.[17]

Many of the aforementioned pathologies are hallmarked by increased leukocyte infiltration. However, intravital microscopy revealed that topical application of LTC_4 to either the mucosa of the hamster cheek pouch or hamster skin caused a profound dose-dependent increase in microvascular permeability but failed to stimulate leukocyte adhesion, suggesting that LTC_4 is unlikely to recruit leukocytes to sites of inflammation.[3,18] These observations are consistent with in vitro findings demonstrating that LTC_4 does not induce neutrophil aggregation and adhesion to subendothelial matrices or other noncellular substrata.[19] Although these data suggest that LTC_4 does not directly affect leukocyte function, a substantial amount of literature would suggest that LTC_4 may contribute significantly to leukocyte recruitment via activation of the endothelium. Treatment of endothelium with cysteinyl leukotrienes increased adhesivity of these cells for leukocytes.[19,20] Further characterization of the increased endothelial adhesivity revealed that LTC_4 caused endothelial cells to rapidly synthesize (within minutes) PAF, a phospholipid that is known to increase leukocyte adhesion by activating the β_2-integrin (CD18). However, while PAF receptor antagonists and monoclonal antibodies directed against CD18 prevented part of the adhesive interaction, there always remained significant PAF- and CD18-independent leukocyte adhesion. On the basis of preliminary anti–P-selectin antibody studies, Zimmerman et al[19] proposed that this additional adhesive mechanism was mediated by P-selectin.

Received May 24, 1995; accepted August 17, 1995.

From the Immunology Research Group, University of Calgary (Canada).

Correspondence to Dr Paul Kubes, Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada.

Selected Abbreviations and Acronyms

NAcLac = *N*-acetyllactosamine
fMLP = f-Met-Leu-Phe
LTC_4, LTD_4, and LTE_4 = leukotrienes C_4, D_4, and E_4, respectively
PAF = platelet-activating factor
sLe^x = sialyl Lewis^x

There is a growing body of evidence to suggest that P-selectin (also known as CD62P and GMP-140), a member of the selectin family of carbohydrate binding proteins, is responsible for a weak, transient, adhesive interaction known as leukocyte rolling, which is a necessary prerequisite for firm adhesion and subsequent emigration.[21-24] P-selectin is stored in Weibel-Palade bodies of endothelial cells and, upon stimulation with such agents as histamine or thrombin, can be rapidly mobilized to the endothelial cell surface.[25] Although the P-selectin–dependent leukocyte–endothelial cell interaction is manifested as adhesion in static assay systems, incorporation of P-selectin into a model membrane supports leukocyte rolling under shear conditions.[21,22] More recent work in vivo has implicated a role for P-selectin as the adhesive moiety that supports leukocyte rolling under normal conditions as well as in various models of inflammation.[26-28] Clearly, if LTC_4-induces P-selectin–dependent leukocyte rolling, then this mediator may be critically involved in leukocyte recruitment during the inflammatory process.

We used intravital microscopy in a rat mesenteric preparation to visualize leukocyte behavior on-line in the presence and absence of LTC_4. Our primary objective was to determine if LTC_4 can indeed induce leukocyte rolling in single 20- to 40-μm postcapillary venules in vivo and to systematically assess the molecular mechanisms involved. We examined whether the LTC_4-dependent leukocyte rolling was mediated by P-selectin and whether the potential P-selectin ligands, L-selectin, or the fucosylated oligosaccharide sLe^x[29] was involved in the rolling interaction. Next, we determined whether leukocyte rolling was dependent on LTC_4 or whether LTC_4 had to be converted to LTD_4 to mediate leukocyte rolling. Finally, we observed that LTC_4 caused leukocyte rolling patterns quite distinct from other P-selectin inducers (histamine) and further characterized this unexpected observation.

Materials and Methods

Intravital Microscopy Experimentation

Male Sprague-Dawley rats (175 to 250 g) were maintained on a purified laboratory diet and fasted 18 to 24 hours before surgery. The animals were initially anesthetized with pentobarbital sodium (65 mg/kg body wt), and a right carotid artery and vein were cannulated to measure systemic arterial blood pressure (Statham P23A transducer and Grass physiological recorder) and drug administration, respectively. All animals were pretreated with sodium cromoglycate (5 mg/kg IV) to prevent baseline leukocyte rolling, as previously described.[28] A midline abdominal incision was made, and a segment of the midjejunum was gently exteriorized and carefully placed over an optically clear viewing pedestal that permitted transillumination of a 2-cm^2 segment of the mesentery. Single unbranched postcapillary venules (20 to 40 μm in diameter) were visualized, and the following parameters were measured: leukocyte rolling, leukocyte rolling velocity, leukocyte adhesion, centerline red blood cell velocity, and shear rates. This preparation has been used extensively by us[28-30,31] and others.[32-34]

Experimental Protocol

Immediately after finding a venule of an appropriate size, the image was recorded for 5 minutes, followed by three additional 5-minute recordings, during which the experimental parameters were assessed. In the first series of experiments, the mesentery was superfused with bicarbonate-buffered saline for the first 5 minutes and then with various concentrations of LTC_4 (0, 2, or 20 nmol/L) for the remaining 55 minutes. Leukocyte rolling and leukocyte rolling velocity were assessed over 60 minutes. Since 20 nmol/L LTC_4 produced the maximum increase in leukocyte rolling, this concentration was used for all subsequent experiments. We directly compared our observations with LTC_4 to a second proinflammatory agonist, histamine (100 μmol/L), which is known to induce significant P-selectin–dependent leukocyte rolling in vivo.[28]

In the next series of experiments, we studied the molecular mechanisms involved in the LTC_4-induced leukocyte rolling. First, we examined a role for sLe^x, a sialylated fucosylated oligosaccharide that is known to bind to the lectin domain of selectins. Animals were pretreated with a soluble form of sLe^x (1 mg/100 g body wt, Alberta Research Council) before LTC_4 exposure, and leukocyte rolling and rolling velocity were assessed. We also examined the effect of a control carbohydrate, NAcLac, which lacks the fucose sugar moiety and the 3′-sialyl group on the galactose residue normally found on sLe^x.

In another series of experiments, animals received an anti–P-selectin antibody, PB1.3 (P-selectin–blocking IgG1-clone 352; Dr James Paulson, Cytel Corp) at 2 mg/kg IV at 15 minutes of LTC_4 exposure. We have previously demonstrated that this concentration of PB1.3 was most effective at preventing P-selectin–dependent leukocyte rolling in vivo.[28] An isotype-matched control antibody had no effect on leukocyte kinetics. To study a role for L-selectin, animals were given an anti–L-selectin antibody, HRL3 (Upjohn Co), at 1 mg/kg IV as previously described.[35,36]

To further characterize the mechanisms underlying the LTC_4-induced leukocyte rolling, we examined the effect of L-serine, which prevents the conversion of LTC_4 to LTD_4 by inhibiting the enzyme, γ-glutamyltranspeptidase.[37] In this series of experiments, L-serine (100 μmol/L) or its inactive enantiomer, D-serine, was superfused over the mesentery for 5 minutes before LTC_4 superfusion, and leukocyte rolling and leukocyte rolling velocity were assessed. In addition, we examined the effect of MK 571 (Merck-Frosst, Canada Inc), a potent LTD_4 receptor antagonist that has been demonstrated to antagonize a wide range of LTC_4-mediated effects in various species.[38] In this series of experiments, animals received MK 571 (30 mg/kg IV) just before LTC_4 superfusion.

The number of adherent leukocytes within a 100-μm segment of venule was also quantified before and after LTC_4 superfusion. It has been demonstrated in vitro that LTC_4 can induce the expression of endothelial cell–associated PAF[20] and thereby support leukocyte adhesion in static assay systems. To study a role for PAF in our model of LTC_4-induced leukocyte rolling and adhesion, we pretreated a group of animals with a PAF receptor antagonist, WEB 2086 (10 mg/kg IV, Boehringer-Ingelheim), and LTC_4-induced leukocyte rolling flux, leukocyte rolling velocity, and leukocyte adhesion were observed over 60 minutes. To determine whether or not the LTC_4-induced reduction in leukocyte rolling velocity increased the propensity of neutrophils to adhere when subsequently exposed to a chemotactic agent, we superfused PAF (1 nmol/L) at a concentration that does not normally cause adhesion of cells rolling at a control velocity. PAF was added to the LTC_4 or the histamine preparation in these experiments, and rolling and adhesion were assessed.

TABLE 1. Hemodynamic Parameters in Untreated and LTC$_4$-Treated Animals at 60 Minutes

	Untreated	LTC$_4$-Treated
Venular diameter, μm	23.2±2.9	25.3±1.2
Red blood cell velocity, mm/s	1.83±0.36	2.07±0.52
Shear rates, s^{-1}	397.2±75.3	407.0±95.6

Values are mean±SEM.

Statistical Analysis

Data are presented as mean±SEM. A one-way ANOVA and Student's *t* test with Bonferroni correction were used for multiple comparisons. Statistical significance was set at $P<.05$.

Results

The baseline hemodynamic parameters at 60 minutes in untreated and LTC$_4$-treated (20 nmol/L) animals are summarized in Table 1. In all animals, venular diameter remained constant over the entire duration of the experimental protocol. Red blood cell velocity and shear rates within the venules of study did not change with time in either the untreated or the experimental group, negating any effects of hemodynamic factors on leukocyte behavior.

Fig 1 demonstrates the flux of rolling leukocytes in response to varying concentrations of LTC$_4$. In untreated animals, the flux of rolling leukocytes remained below 15 cells per minute throughout the entire experiment. A low concentration (2 nmol/L) of LTC$_4$ superfusion induced a small increase in the flux of rolling leukocytes that reached significance only at the early time point (15 minutes). LTC$_4$ at 20 nmol/L, however, caused a pronounced increase in leukocyte rolling that was maintained for the entire duration of the experiment. Interestingly, in addition to the increased number of rolling leukocytes, the average velocity of rolling leukocytes was greatly reduced (Fig 2), so that the cells appeared to be "creeping" along the length of the venule. The surface area of contact between the rolling leukocytes and the endothelium appeared to be greatly increased relative to leukocytes rolling in the absence of

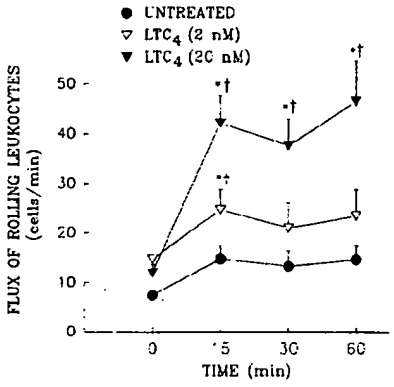

FIG 1. Effect of varying concentrations of LTC$_4$ on the flux of rolling leukocytes over 60 minutes. In untreated animals (n=6), there is no increase in leukocyte rolling flux over time. LTC$_4$ at a concentration of 2 nmol/L induced an increase in leukocyte rolling flux at 15 minutes only (n=4), whereas 20 nmol/L LTC$_4$ induced a significant and sustained increase in the flux of rolling leukocytes (n=5). *$P<.05$ relative to 0-minute value. †$P<.05$ relative to respective untreated value.

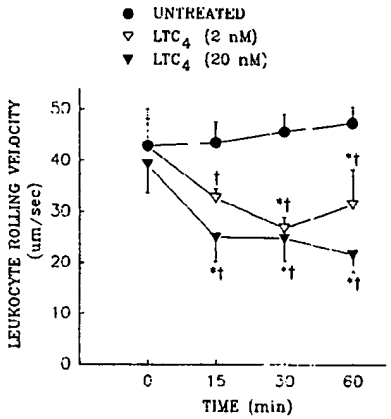

FIG 2. Changes in leukocyte rolling velocity elicited by exogenously administered LTC$_4$ (20 nmol/L). LTC$_4$ induced a rapid and significant reduction in leukocyte rolling velocity (n=5) that was maintained for the entire duration of the experiment. Rolling velocity remained unchanged for 60 minutes in untreated controls (n=6). *$P<.05$ relative to 0-minute value. †$P<.05$ relative to respective untreated value.

LTC$_4$. It should also be noted that at 2 nmol/L LTC$_4$, the few new rolling leukocytes (at 15 minutes) and the few baseline rolling leukocytes (throughout the 60-minute period) rolled at a slower velocity. This was the first evidence that rolling velocity could be dissociated from the actual recruitment of rolling leukocytes. The LTC$_4$-induced reduction in rolling velocity was independent of any hemodynamic alterations, as red blood cell velocity and shear rates remained unchanged during LTC$_4$ superfusion (Table 1).

The increased flux of rolling leukocytes and decreased rolling velocity were compared directly to the leukocyte rolling response elicited with 5000 times the concentration of histamine (Fig 3). This concentration has previously been shown to be optimal for recruitment of rolling leukocytes.[28] Histamine administration at a dose of 100 μmol/L induced a significant increase in leukocyte rolling (Fig 3, top) but, unlike LTC$_4$ (at either 2 or 20 nmol/L), had absolutely no effect on leukocyte rolling velocity (Fig 3, bottom). Furthermore, even when histamine concentration was increased to 1 mmol/L, leukocyte rolling velocity remained unchanged (data not shown), suggesting that LTC$_4$ and histamine exert distinct leukocyte rolling profiles.

Fig 4 summarizes the results for sLe^x on the LTC$_4$-induced increase in leukocyte rolling flux and reduction in leukocyte rolling velocity. Pretreatment of animals with a soluble form of sLe^x prevented the LTC$_4$-induced increase in leukocyte rolling flux (Fig 4, top). Moreover, sLe^x pretreatment also prevented the LTC$_4$-induced reduction in leukocyte rolling velocity (Fig 4, bottom). In fact, the few remaining leukocytes that rolled after sLe^x administration did so at a velocity that was above baseline. A control carbohydrate, NAcLac, did not inhibit the flux of rolling leukocytes; however, at 60 minutes, it did ablate the LTC$_4$-induced reduction in leukocyte rolling velocity (44.4±8.8 μm/s with NAcLac pretreatment compared with 21.7±3.5 μm/s with LTC$_4$ alone).

We further examined the mechanisms of LTC$_4$-induced leukocyte rolling by testing a P-or L-selectin

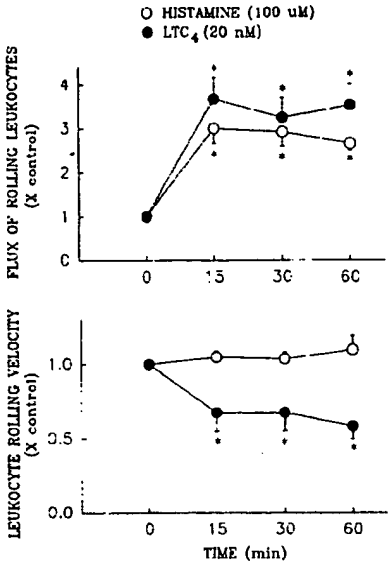

FIG 3. A comparison of the effects of histamine (100 μmol/L, n=4) and LTC_4 (20 nmol/L, n=5) on leukocyte rolling flux (top) and leukocyte rolling velocity (bottom). Histamine administration at 100 μmol/L induced a similar increase in the flux of rolling leukocytes as LTC_4; however, LTC_4 was administered at <5000 times the concentration of histamine. Histamine at this concentration had absolutely no effect on leukocyte rolling velocity (bottom), whereas LTC_4 significantly reduced rolling velocity. *$P<.05$ relative to 0-minute value.

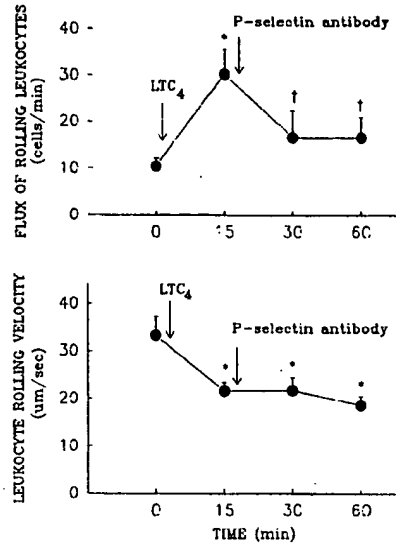

FIG 5. Effect of an anti-P-selectin antibody on the LTC_4-induced increase in leukocyte rolling (n=4, top) and decrease in leukocyte rolling velocity (n=4, bottom). Administration of an anti-P-selectin antibody at 20 minutes immediately reversed the LTC_4-induced increase in leukocyte rolling but had no effect on the reduction in rolling velocity. *$P<.05$ relative to 0-minute value. †$P<.05$ relative to 15-minute value.

antibody. Fig 5, top, illustrates that the anti-P-selectin antibody PB1.3 reversed the LTC_4-induced increase in the flux of rolling leukocytes, an effect that was maintained for the remainder of the experiment, even in the presence of continued LTC_4 superfusion. Fig 5, bottom, demonstrates that PB1.3 had no effect on the LTC_4-induced reduction in rolling velocity, suggesting that P-selectin did not contribute to the reduced velocity with which the remaining few leukocytes rolled. Fig 6 illustrates the effect of anti-L-selectin antibody HRL3 on the LTC_4-induced leukocyte rolling and reduction in rolling velocity. HRL3 administration, either as a pretreatment (data not shown) or as a posttreatment, had no effect on

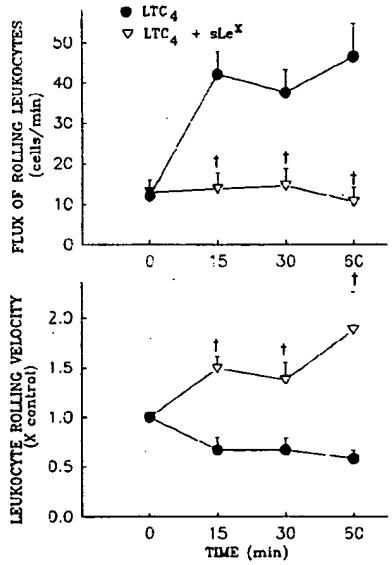

FIG 4. Effect of sLe^x pretreatment on the LTC_4-induced increase in leukocyte rolling flux (top) and decrease in rolling velocity (bottom). sLe^x pretreatment inhibited both the LTC_4-induced increase in the flux of rolling leukocytes and the LTC_4-induced reduction in rolling velocity (n=4). In fact, sLe^x pretreatment increased leukocyte rolling velocity above baseline values. †$P<.05$ relative to respective LTC_4 value.

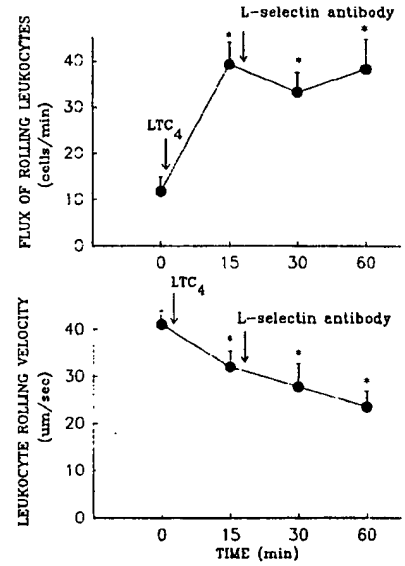

FIG 6. Effect of an anti-L-selectin antibody on the LTC_4-induced leukocyte rolling and reduction in rolling velocity. An anti-L-selectin antibody administered at 20 minutes of the experimental protocol had no effect on the LTC_4-induced increase in leukocyte rolling (n=4, top). The L-selectin antibody also had no effect on the LTC_4-induced reduction in rolling velocity (n=4, bottom). *$P<.05$ relative to 0-minute value.

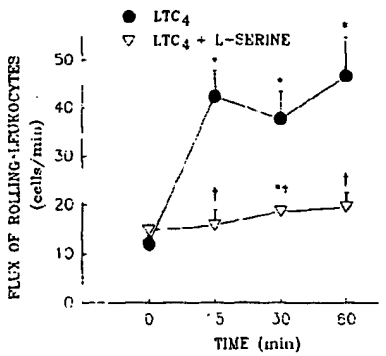

FIG 7. Effect of L-serine pretreatment on the LTC$_4$-induced increase in leukocyte rolling flux. Inhibition of LTC$_4$ conversion to LTD$_4$ by L-serine abolished the LTC$_4$-induced increase in leukocyte rolling flux (n=5). *P<.05 relative to 0-minute value. †P<.05 relative to respective LTC$_4$ value.

the LTC$_4$-induced increase in leukocyte rolling flux (Fig 6, top) or the LTC$_4$-induced reduction in rolling velocity (Fig 6, bottom). These data suggest that L-selectin is not involved in LTC$_4$-induced P-selectin–dependent alterations in leukocyte behavior. HRL3, at the same concentration, did reduce leukocyte rolling in other inflammatory models.[35,36]

L-Serine pretreatment (which inhibits γ-glutamyltranspeptidase and thereby prevents the conversion of LTC$_4$ to LTD$_4$) completely inhibited both the LTC$_4$-induced increase in the flux of rolling leukocytes (Fig 7) and the LTC$_4$-induced reduction in rolling velocity (data not shown). These observations suggest that the LTC$_4$-induced leukocyte rolling is mediated via the LTD$_4$ receptor. D-Serine pretreatment had no effect on either parameter (data not shown). The effects of MK 571, an LTD$_4$ receptor antagonist, was much less impressive in reducing LTC$_4$-induced leukocyte rolling. In fact, MK 571 pretreatment of animals only attenuated the LTC$_4$-induced increase in leukocyte rolling flux for the first 15 minutes (data not shown). A 10-fold greater increase in MK 571 did not further affect the LTC$_4$-induced leukocyte responses.

Table 2 summarizes changes in leukocyte rolling flux, leukocyte rolling velocity, and leukocyte adhesion under control conditions (0 minutes) and after 60 minutes of exposure to LTC$_4$ in the absence and presence of WEB 2086. WEB 2086 had no effect on leukocyte rolling flux in untreated animals or in animals exposed to LTC$_4$. The LTC$_4$-induced reduction in leukocyte rolling velocity, however, was inhibited by WEB 2086 pretreatment. LTC$_4$ induced a slight, yet significant, increase in leukocyte adhesion, which was significantly attenuated with WEB 2086 pretreatment. These observations suggest that PAF may be involved in the reduction in leukocyte rolling velocity and the small increase in adhesion observed with LTC$_4$.

To determine whether the reduction in rolling velocity facilitates leukocyte adhesion, animals were treated with either histamine or LTC$_4$ and then exposed to the chemotactic agent PAF (1 nmol/L). PAF superfusion induced a significantly greater increase in leukocyte adhesion with LTC$_4$ when compared with animals treated with histamine. Histamine, more so than LTC$_4$, induces the endogenous production of PAF, which may contribute to the subsequent desensitization of PAF receptors on leukocytes.[19] Therefore, to ensure that the lack of response to exogenous PAF with histamine was not simply specific for PAF, we used a second exogenous chemoattractant, fMLP (10 nmol/L). The data revealed that fMLP induced many more rolling leukocytes to adhere with LTC$_4$ exposure than with histamine exposure (data not shown). These data suggest that the LTC$_4$-induced slow rolling cells are more likely to adhere than the faster rolling cells associated with histamine. In both groups, the flux of rolling leukocytes was the same (data not shown).

Discussion

It is generally accepted that under normal conditions there is little leukocyte rolling through postcapillary venules. However, at the time of surgical preparation that is necessary to visualize leukocyte behavior in vivo, there is a dramatic increase in leukocyte rolling.[39] It is well documented that baseline rolling is induced in invasive preparations associated with the mesentery, cheek pouch, and cremaster microcirculation. P-selectin appears to be an important component of baseline rolling, inasmuch as inhibition of P-selectin with selective antibodies[26] or various P-selectin binding moieties[24] or by knocking out the gene for P-selectin in mice[27] decreases baseline rolling. These observations implicate P-selectin in baseline rolling, but at the same time, the artificially elevated level of P-selectin–dependent leukocyte rolling after surgery does not allow for further induction of leukocyte rolling in response to proinflammatory agonists. Therefore, it is not surprising that despite the fact that various potential P-selectin inducers, including histamine and LTC$_4$, have been previously studied by use of intravital microscopy, changes in leukocyte behavior were not noted despite profound changes in other microvascular parameters.[40] We recently developed a model that circumvented the problem of high baseline rolling by identifying mast cell degranulation as a major contributor to the high background rolling.[28] Stabilization of the mast cells before any surgical manipulation significantly reduced baseline rolling, and this has allowed us to investigate potential mediators of P-selectin in vivo. Alternative approaches

TABLE 2. Effect of PAF Receptor Antagonist on LTC$_4$-Induced Alterations in Leukocyte Kinetics

	Control	LTC$_4$	WEB 2086	LTC$_4$+WEB 2086
Leukocyte rolling flux, cells/min	12.0±1.6	46.5±8.1*	13.5±2.3	22.3±9.7
Leukocyte rolling velocity, μm/s	39.3±5.6	21.7±3.5*	38.8±2.4	37.3±7.7†
Leukocyte adhesion, n	1.0±0.4	5.0±1.1*	1.75±1.0	1.3±1.3†

n indicates the number of adherent cells in a 100-μm segment of postcapillary venule. Values are mean±SEM.
*P<.05 relative to control value, †P<.05 relative to LTC$_4$ value.

include either the use of animals with very low baseline rolling[41] or the examination of leukocyte rolling immediately upon exteriorization of the mesentery.[42]

Our results demonstrate that LTC_4 rapidly increases leukocyte rolling via a P-selectin–dependent event. This contention is based on the observation that increased leukocyte rolling was completely reversed by the administration of an anti–P-selectin antibody despite continuous LTC_4 superfusion. These results are entirely consistent with the view that P-selectin can be rapidly (within minutes) mobilized to the endothelial cell surface in response to various proinflammatory mediators, including histamine, thrombin, and oxidants.[25] Although LTC_4 has also been postulated to induce P-selectin expression,[19] data to support this view have not been reported to date. Our data for the first time demonstrate a functional role for P-selectin in vivo after exposure of the microvasculature to LTC_4. This work also suggests that LTC_4 may be far more effective at inducing P-selectin than are mediators such as histamine. In the present study, we demonstrate that LTC_4, at <5000 times the concentration of histamine, induced an equivalent increase in leukocyte rolling. A possible explanation for the greater apparent sensitivity of the endothelium to LTC_4 versus histamine may be the high plasma levels of endogenous histaminase, which might greatly reduce the amount of intact histamine that contacts the endothelium.

Early work proposed that L-selectin may be the endogenous ligand for P-selectin. This was based on the finding that L-selectin antibodies prevented neutrophil binding to P-selectin–transfected COS cells.[43] Our observations do not support this hypothesis; a rat monoclonal antibody to L-selectin did not prevent the P-selectin–dependent (LTC_4-induced) leukocyte rolling in the rat mesenteric microcirculation. This monoclonal antibody does reduce leukocyte rolling in more chronic models of inflammation[36] but clearly is not important in P-selectin–dependent leukocyte rolling. This is in agreement with more recent data suggesting that L-selectin is unlikely to be the ligand for P-selectin in vivo. For example, Nolte et al[44] recently demonstrated that P-selectin is essential for leukocyte rolling in the mouse cremaster and skin, whereas L-selectin appeared not to play a significant role. Ley et al[45] observed that L-selectin–deficient mice had significant baseline rolling, whereas there was a lack of rolling in P-selectin–deficient mice in the early phase of the experimental protocol. These data support a role for P-selectin, but not L-selectin, in the early leukocyte rolling event in postcapillary venules.

A potential ligand for P-selectin may be a fucosylated oligosaccharide, sLe^x, or a closely related sugar,[29] displayed by more complex glycoprotein structures, including P-selectin glycoprotein ligand-1.[46] Zhou et al[29] demonstrated that HL60 cells and Chinese hamster ovary cell lines transfected with α-1.3/4-fucosyltransferase to express sLe^x bound avidly to P-selectin. Our data strongly support this hypothesis; soluble sLe^x entirely prevented the LTC_4-induced P-selectin–dependent increase in leukocyte rolling. These data are consistent with the work of Asako et al,[41] who reported that the rise in histamine-induced leukocyte rolling (P-selectin dependent) could be inhibited with soluble sLe^x. Moreover, Mulligan et al[47] reported that soluble sLe^x reduced P-selectin–dependent neutrophil recruitment and lung injury associated with cobra venom factor, further supporting a role for P-selectin–sLe^x interactions in postcapillary venules.

A very obvious and consistent finding in the present study was that LTC_4 induced a very significant reduction in leukocyte rolling velocity. Qualitatively, rolling leukocytes exposed to LTC_4-treated endothelium had greater surface area attachment to endothelium than did leukocytes rolling under control conditions or those exposed to histamine. These cells appeared to be "crawling" or "creeping" rather than rolling. The reduction in leukocyte rolling velocity occurred without a change in the hydrodynamic dispersal forces (shear forces) that tend to push leukocytes along the length of postcapillary venules, suggesting the involvement of an adhesive mechanism rather than a simple reduction in shear. The data in the present study would suggest that LTC_4 induced a unique adhesive interaction, independent of P-selectin but entirely dependent on sLe^x. Clearly, sLe^x may serve as a ligand for P-selectin to induce rolling (flux) but also may serve as a ligand for an unidentified adhesion molecule to reduce the rolling velocity. It is noteworthy that a very similar slow leukocyte rolling profile has been observed in vitro on E-selectin but not P-selectin.[48] It is also well known that E-selectin binds avidly to sLe^x, perhaps supporting the notion of E-selectin as a potential ligand. However, all of the data to date would suggest that E-selectin is not induced rapidly on endothelium and therefore is an unlikely candidate in the present study.

Although almost no attention has been given to the role of adhesion molecules responsible for leukocyte rolling velocity, this event may be functionally just as important as leukocyte rolling flux. For example, reduced leukocyte rolling velocity may give rolling leukocytes a higher propensity to adhere in the presence of an appropriate stimulus. In fact, the present data support this hypothesis, inasmuch as 1 nmol/L PAF superfusion induced a significantly greater increase in leukocyte adhesion in the presence of LTC_4 (when the rolling leukocytes were rolling very slowly) but not histamine, which increased the number of rolling cells but had no effect on leukocyte rolling velocity. Therefore, simply targeting the molecules responsible for leukocyte rolling velocity may be as effective at inhibiting subsequent adhesion and vascular dysfunction as preventing leukocyte rolling per se.

Although it is tempting to conclude that the reduced rolling velocity with LTC_4 (not histamine) is the reason for the greater adhesive response to PAF, other explanations exist. It is known from in vitro studies that both histamine and LTC_4 induce PAF production from endothelial cells, with greater amounts of PAF produced with histamine than with LTC_4.[49] This PAF remains cell-associated and may contribute to PAF receptor desensitization on leukocytes.[19] Therefore, another explanation for the differences between histamine and LTC_4 depicted in Fig 8 may be that histamine is more effective at causing desensitization of the PAF receptor, thereby blunting the response to exogenously administered PAF. However, observations from our laboratory with another exogenous chemotactic agent, fMLP, would not support this hypothesis. A low dose of fMLP (10 nmol/L) also induces greater leukocyte adhesion in animals exposed

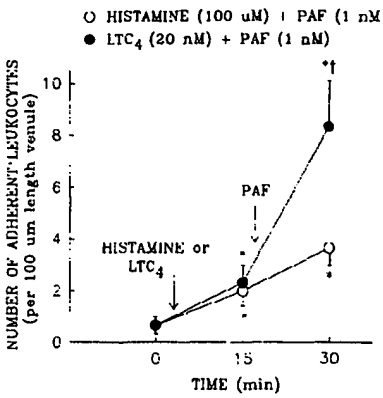

FIG 8. Alterations in leukocyte adhesion in response to a chemotactic factor in animals previously exposed to either histamine (100 μmol/L) or LTC$_4$ (20 nmol/L). PAF (1 nmol/L) superfusion induced an increase in leukocyte adhesion in both groups; however, the PAF-induced leukocyte adhesion was significantly greater in animals exposed to LTC$_4$ compared with the histamine-treated group. *P<.05 relative to 0-minute value. †P<.05 relative to respective histamine value.

to LTC$_4$ compared with those treated with histamine, suggesting that the observation is not particular to PAF. Finally, another explanation for the results in Fig 8 could be that histamine may be more potent than LTC$_4$ at inducing the generation of an anti-inflammatory agonist (eg, nitric oxide or prostacyclin), which would then blunt the response to subsequent exposure to chemotactic stimuli. This possibility warrants further attention.

Previous in vitro work has demonstrated that LTC$_4$-induced PAF synthesis on the surface of endothelium promotes CD18-dependent leukocyte adhesion.[19] Although one might predict from these in vitro experiments a significant increase in leukocyte adhesion in postcapillary venules treated with LTC$_4$, the magnitude of the LTC$_4$-induced adhesion in the present study was subtle compared with that observed with exogenous PAF or various other chemotactic agents.[50] When animals were pretreated with WEB 2086, not only was the LTC$_4$-induced adhesion decreased, but the rolling velocity also returned to baseline levels. These data suggest that PAF may also be involved in the reduced rolling velocity associated with LTC$_4$. This is consistent with observations that WEB 2086 inhibits the reduction in leukocyte rolling velocity and adhesion in various inflammatory conditions, including ischemia/reperfusion and mast cell–dependent leukocyte recruitment.[51,52]

Once released from a cell, LTC$_4$ is rapidly and predominantly metabolized to LTD$_4$ via γ-glutamyltranspeptidase and then to LTE$_4$ via aminopeptidase.[37,53,54] Depending on the species and route of elimination, very small amounts of LTC$_4$ are actually detectable systemically.[1,55,56] In humans, urinary levels of LTD$_4$ and LTE$_4$ are often used as estimates of systemic LTC$_4$ generation in pathophysiological states, including inflammatory bowel disease, glomerulonephritis, and rheumatoid arthritis.[12,57,58] In animal models, investigators have primarily studied the effect of LTC$_4$ on bronchial smooth muscle contraction and microvascular permeability in lung tissue and have demonstrated that although selective receptors for LTC$_4$ may exist,[38,53,59] the majority of the actions of LTC$_4$ are in fact mediated via the LTD$_4$ receptor.[38,54] In the present study, we demonstrate that inhibition of the LTC$_4$ bioconversion to LTD$_4$ and the subsequent bioconversion to LTE$_4$ prevent the LTC$_4$-induced increase in leukocyte rolling and reduction in rolling velocity, suggesting that these responses are indeed mediated via the LTD$_4$ receptor. However, the possibility that an LTE$_4$ receptor exists and mediates this response cannot be excluded. The reduction in LTC$_4$-induced leukocyte rolling with L-serine was as effective as that with sLe^x and raises the possibility that targeting the enzyme responsible for LTC$_4$ conversion to LTD$_4$ (γ-glutamyltranspeptidase) may be a rational approach to anti-inflammatory therapy. A specific LTD$_4$ receptor antagonist did not entirely prevent the LTC$_4$-induced leukocyte–endothelial cell interactions; however, this may be explained by the fact that LTD$_4$ receptors display significant species specificity.[60-63]

In conclusion, our data provide the first evidence that LTC$_4$ can indeed induce leukocyte rolling in postcapillary venules via a P-selectin–dependent and sLe^x-dependent mechanism. These events are likely to be mediated by LTC$_4$ bioconversion to LTD$_4$. Moreover, LTC$_4$ induced a rapid and significant sLe^x-dependent reduction in leukocyte rolling velocity, which further increased the likelihood that a rolling leukocyte would adhere.

Acknowledgments

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            $0.35    0.099 DialUnits File1
     $0.35  Estimated cost File1
     $0.35  Estimated cost this search
     $0.35  Estimated total session cost   0.099 DialUnits

File 410:Chronolog(R)  1981-2002/Nov
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HILIGHT set on as ''
HILIGHT set on as ''
? begin 5,73,155,399
       23dec02 08:21:44 User208760 Session D2234.2
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     $0.00  Estimated cost File410
     $0.01  TELNET
     $0.01  Estimated cost this search
     $0.36  Estimated total session cost   0.170 DialUnits

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      Set  Items  Description
      ---  -----  -----------
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? s ltc4 and (thrombus or thrombotic)
            4556  LTC4
           43193  THROMBUS
           37470  THROMBOTIC
      S1       7  LTC4 AND (THROMBUS OR THROMBOTIC)
? rd s1
...completed examining records
      S2       4  RD S1 (unique items)
? t s2/3/all

 2/3/1     (Item 1 from file: 5)
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07396805   BIOSIS NO.: 000091012415
TRANSCELLULAR BIOSYNTHESIS OF SULFIDOPEPTIDE LEUKOTRIENES DURING
  RECEPTOR-MEDIATED STIMULATION OF HUMAN NEUTROPHIL-PLATELET MIXTURES
AUTHOR: MACLOUF J; MURPHY R C; HENSON P M
AUTHOR ADDRESS: NATIONAL JEWISH CENTER IMMUNOLOGY RESPIRATORY MEDICINE,
  1400 JACKSON ST., DENVER, COLO. 80206.
JOURNAL: BLOOD 76 (9). 1990. 1838-1844. 1990
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH


 2/3/2     (Item 2 from file: 5)
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06782935   BIOSIS NO.: 000088092372
TRANSCELLULAR SULFIDOPEPTIDE LEUKOTRIENE BIOSYNTHETIC CAPACITY OF VASCULAR
  CELLS
AUTHOR: MACLOUF J; MURPHY R C; HENSON P M
AUTHOR ADDRESS: DEP. PHARMACOL. C236 , UNIV. COLO. HEALTH SCI. CENT., 4200
  EAST 9TH AVENUE, DENVER, COLO. 80262.
JOURNAL: BLOOD 74 (2). 1989. 703-707. 1989
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH


 2/3/3     (Item 3 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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06232666   BIOSIS NO.: 000086066848
SYNERGISTIC EFFECTS OF LTC-4 AND TXA-2 ON CORONARY FLOW AND MYOCARDIAL
  FUNCTION
AUTHOR: NICHOLS W W; MEHTA J L; THOMPSON L; DONNELLY W H
AUTHOR ADDRESS: DEP. MED., UNIV. FLORIDA COLL. MED., GAINESVILLE, FLA.
  32610.
JOURNAL: AM J PHYSIOL 255 (1 PART 2). 1988. H153-H159. 1988
FULL JOURNAL NAME: American Journal of Physiology
CODEN: AJPHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH


 2/3/4     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08020785 94167673 PMID: 8122190
Intravascular cysteinyl-leukotriene formation by clotting whole human blood. Evidence from clamped umbilical vein segments and **thrombus** specimens.
Weide I; Winking M; Simmet T
Department of Pharmacology and Toxicology, Ruhr University, Bochum, Germany.
Thrombosis research (UNITED STATES) Oct 1 1993, 72 (1) p83-90,
ISSN 0049-3848 Journal Code: 0326377
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
? t s2/7/all

2/7/1 (Item 1 from file: 5)

07396805 BIOSIS NO.: 000091012415
TRANSCELLULAR BIOSYNTHESIS OF SULFIDOPEPTIDE LEUKOTRIENES DURING RECEPTOR-MEDIATED STIMULATION OF HUMAN NEUTROPHIL-PLATELET MIXTURES
AUTHOR: MACLOUF J; MURPHY R C; HENSON P M
AUTHOR ADDRESS: NATIONAL JEWISH CENTER IMMUNOLOGY RESPIRATORY MEDICINE, 1400 JACKSON ST., DENVER, COLO. 80206.
JOURNAL: BLOOD 76 (9). 1990. 1838-1844. 1990
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The ability of different cell types to cooperate in the metabolism of reactive intermediates of arachidonic acid such as leukotriene A4 (LTA4) is currently receiving considerable attention. Of critical importance is the demonstration that transfer of LTA4 could occur under conditions when relatively low amounts of LTA4 are produced such as would be expected for in vitro receptor-mediated stimulation. Stimulation of human neutrophils with a combination of chemotactic factor (formyl-methionyl-leucyl-phenylalanine, FMLP) and phagocytosable particles (opsonized zymosan) resulted in little production of **LTC4** alone, but measurable quantities appeared when platelets were coincubated. When these agonists were added to platelets alone in the absence of neutrophils, no **LTC4** was produced. In the presence of stimulated neutrophils, the final synthesis of **LTC4** was shown to occur within the platelets (from neutrophil-derived by LTA4) by experiments using platelets that had been prelabeled with 35S-cysteine to label intracellular platelet glutathione. Other 35S-labeled sulfidopeptide leukotriene metabolites were also produced in this coincubation of neutrophils and platelets. The observed synergy between FMLP and opsonized zymosan in the production of **LTC4** when neutrophils and platelets were coincubated may involve priming the neutrophil for LTA4 production. Activation of platelets or endothelial cells with thrombin did not alter the capacity of either cell to convert exogenously added LTA4 to **LTC4**. This would support the suggestion that even when platelets are activated they retain their capacity to metabolize LTA4 into **LTC4**. Finally, previous exposure of the platelets to LTA4 did not affect subsequent metabolism of arachidonic acid by the cyclooxygenase pathway to thromboxane A2 (TXA2) except at very high concentration of LTA4. These results suggest that cell-cell interactions may be critical determinants of the profile of eicosanoids produced by physiologic and pathophysiologic circumstances. In particular, we believe that both endothelial cells and platelets can, together with neutrophils, contribute relatively large amounts of sulfidopeptide leukotrienes to inflammatory and **thrombotic** events.

These cell-cell interactions are aspirin-insensitive; therefore, aspirin-treated platelets are capable of synthesizing the vasoconstrictor **LTC4** from neutrophil LTA4 at a time when they can no longer produce thromboxane.

2/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06782935 BIOSIS NO.: 000088092372
TRANSCELLULAR SULFIDOPEPTIDE LEUKOTRIENE BIOSYNTHETIC CAPACITY OF VASCULAR CELLS
AUTHOR: MACLOUF J; MURPHY R C; HENSON P M
AUTHOR ADDRESS: DEP. PHARMACOL. C236 , UNIV. COLO. HEALTH SCI. CENT., 4200 EAST 9TH AVENUE, DENVER, COLO. 80262.
JOURNAL: BLOOD 74 (2). 1989. 703-707. 1989
FULL JOURNAL NAME: Blood
CODEN: BLOOA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Cells in the vasculature, including polymorphonuclear leukocytes, platelets, endothelial cells, have been shown to be jointly involved in the biosynthesis of active lipid mediators derived from arachidonic acid. Stimulation of neutrophils with the calcium ionophore A23187 as a model for cell activation results in production of leukotriene (LT)A4 with subsequent intracellular conversion into LTB4. When platelets or endothelial cells were present in the incubation system, **LTC4** was produced from the neutrophil-derived LTA4. Whereas production and release of LTA4 under resting conditions in vivo might be expected to be quite low, under pathologic conditions, LTA3 production could be markedly increased. Therefore, the metabolism of exogenous LTA4 by platelets and endothelial cells was studied at a wide range of LTA4 concentrations. The production of **LTC4** during coincubation of neutrophils with platelets was found to be dependent on neutrophil number ranging from 2 .times. 105 to 2 .times. 107 cells/mL. When a fixed number of neutrophils were stimulated with platelets alone or with mixtures of platelets and endothelial cells, **LTC4** production was observed to be dependent on both acceptor cell types. These results suggest that mixed cell populations, which are likely to occur in vivo, may be critical determinants of the profile of eicosanoids produced in pathophysiologic circumstances. We suggest that both endothelial cells and platelets, in the presence of neutrophils, contribute large quantities of sulfidopeptide leukotrienes to inflammatory and **thrombotic** situations. Furthermore, platelets, because of their quantity and reactivity, may play a pivotal role in transcellular biosynthesis of eicosanoids.

2/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06232666 BIOSIS NO.: 000086066848
SYNERGISTIC EFFECTS OF LTC-4 AND TXA-2 ON CORONARY FLOW AND MYOCARDIAL FUNCTION
AUTHOR: NICHOLS W W; MEHTA J L; THOMPSON L; DONNELLY W H
AUTHOR ADDRESS: DEP. MED., UNIV. FLORIDA COLL. MED., GAINESVILLE, FLA. 32610.
JOURNAL: AM J PHYSIOL 255 (1 PART 2). 1988. H153-H159. 1988
FULL JOURNAL NAME: American Journal of Physiology
CODEN: AJPHA
RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Accumulation of leukocytes and platelets in the injured myocardium may influence coronary arteriolar resistance via release of peptidoleukotrienes and thromboxane A2 (TxA2). Although leukotriene C4 (**LTC4**) and TxA2 individually reduce coronary blood flow, the effects of their simultaneous administration are not known. Accordingly, the combined effects of intracoronary administration of synthetic **LTC4** and thromboxane "mimic" U 46619 on coronary blood flow in patent and severely narrowed coronary arteries and myocardial segmental function were examined in eight anesthetized dogs. Administration of **LTC4** and U 46619 (0.3-3 .mu.g) showed concentration-dependent reductions in coronary blood flow and myocardial segment shortening. When these eicosanoids (3 .mu.g each) were administered in close sequence, coronary blood flow decreased 33% in the patent artery and 47% in the narrowed artery. This reduction in coronary blood flow in the narrowed coronary artery produced a 26% decrease in myocardial segment shortening. Reductions in narrowed coronary artery blood flow and myocardial segment shortening were greater than the sum of changes caused by **LTC4** and U 46619 administered separately ($P < 0.05$). The histopathology of the narrowed coronary artery revealed endothelial disruption at the site of occlusion and an in vivo **thrombus** just distal to the site of occlusion. These data suggest marked synergism between the actions of **LTC4** and TxA2, which may be released locally at the site of coronary **thrombus**, on blood flow in a severely narrowed coronary artery, and on segmental function in the region supplied by this vessel.

2/7/4 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08020785 94167673 PMID: 8122190

Intravascular cysteinyl-leukotriene formation by clotting whole human blood. Evidence from clamped umbilical vein segments and **thrombus** specimens.

Weide I; Winking M; Simmet T

Department of Pharmacology and Toxicology, Ruhr University, Bochum, Germany.

Thrombosis research (UNITED STATES) Oct 1 1993, 72 (1) p83-90, ISSN 0049-3848 Journal Code: 0326377

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have recently demonstrated that contact activation of the intrinsic coagulation cascade in vitro is accompanied not only by thromboxane (TX) B2 generation but also by the formation of 5-lipoxygenase-derived cysteinyl-leukotrienes (LT). In our present study we have investigated the effects of the vascular wall on the eicosanoid formation by whole human blood. Incubation of whole human blood in clamped segments of autologous umbilical veins incubated in oxygenated Tyrode solution led to a time-dependent generation of cysteinyl-LT and TXB2 in the blood samples. A clear dissociation in the time-dependent production profiles was observed with cysteinyl-LT practically reaching a plateau phase at 60 min while TXB2 levels increased up to 90 min. In blood samples incubated in glass tubes for 60 min TXB2 production was about 13 times higher and cysteinyl-LT formation only about half as much as in the umbilical vein segments indicating a differential stimulation of both the cyclooxygenase and 5-lipoxygenase pathway of arachidonic acid metabolism in these experiments. By reverse phase HPLC the immunoreactive cysteinyl-LT were identified as a mixture of **LTC4** , LTD4 and LTE4. Since the data were suggestive of intravascular cysteinyl-LT formation in **thrombotic** vessels, **thrombus** specimens from patients with acute deep vein thrombosis of the lower limb were analysed for these compounds by combined reverse phase

HPLC and specific radioimmunoassay.(ABSTRACT TRUNCATED AT 250 WORDS)
Record Date Created: 19940407

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? s ltc4 and (p(w)selectin)
            4556  LTC4
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           28903  SELECTIN
           10874  P(W)SELECTIN
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...completed examining records
      S4      11  RD S3 (unique items)
? t s3/7/all
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3/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12977323 BIOSIS NO.: 200100184472
Soluble **P-selectin** antagonist mediates rolling velocity and adhesion of leukocytes in acutely inflamed venules.
AUTHOR: Eppihimer Michael J(a); Schaub Robert G
AUTHOR ADDRESS: (a)Discovery Research: Immunology and Hemostasis, Wyeth/Genetics Institute, Inc., One Burtt Road, Andover, MA, 01810: meppihimer@genetics.com**USA
JOURNAL: Microcirculation (New York) 8 (1):p15-24 February, 2001
MEDIUM: print
ISSN: 1073-9688
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Objective: Leukocyte rolling is recognized as an important event in facilitating the extravasation of leukocytes from the vascular to the interstitial compartment, and is mediated by the selectin family of cell adhesion molecules. The aim of this study was to evaluate and characterize the rolling behavior of leukocytes in a model of acute inflammation using a novel soluble selectin ligand directed against **P-selectin**. Methods: Feline mesenteric postcapillary venules were visualized using intravital microscopy prior to and following exposure to leukotriene C4 (**LTC4**) in animals pretreated with vehicle (saline) and the **P-selectin** antagonist rPSGL-Ig. Results: A concentration of 500 pM **LTC4** induced a threefold and sixfold elevation in leukocyte rolling flux and adhesion, respectively, compared to baseline values ($p < 0.05$). Administration of rPSGL-Ig had no effect on **LTC4**-induced leukocyte rolling flux but significantly attenuated the increase in the fraction of rolling leukocytes ($p < 0.05$). In addition, rPSGL-Ig inhibited the **LTC4**-induced reductions in leukocyte rolling velocity ($p < 0.001$). Finally, **LTC4**-induced leukocyte adhesion in animals pretreated with rPSGL-Ig was reduced by 60%, compared to vehicle-treated animals ($p < 0.05$). Conclusions: **LTC4** induces leukocyte rolling and adhesion in feline mesenteric venules in a dose-dependent manner. Administration of rPSGL-Ig inhibits **LTC4**-induced reductions in leukocyte rolling velocity and attenuates the elevation in the fraction of rolling leukocytes produced by **LTC4** stimulation. This suggests that rPSGL-Ig may be used to reduce leukocyte rolling and adhesion, and subsequently attenuate tissue injury during inflammation.

3/7/2 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11163195 21190512 PMID: 11296849

Soluble **P-selectin** antagonist mediates rolling velocity and adhesion of leukocytes in acutely inflamed venules.

Eppihimer M J; Schaub R G
Wyeth/Genetics Institute, Andover, MA 01810, USA. meppihimer@genetics.com

Microcirculation (New York, N.Y. : 1994) (United States) Feb 2001, 8 (1) p15-24, ISSN 1073-9688 Journal Code: 9434935
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

OBJECTIVE: Leukocyte rolling is recognized as an important event in facilitating the extravasation of leukocytes from the vascular to the interstitial compartment, and is mediated by the selectin family of cell adhesion molecules. The aim of this study was to evaluate and characterize the rolling behavior of leukocytes in a model of acute inflammation using a novel soluble selectin ligand directed against **P-selectin**.
METHODS: Feline mesenteric postcapillary venules were visualized using intravital microscopy prior to and following exposure to leukotriene C4 (**LTC4**) in animals pretreated with vehicle (saline) and the **P-selectin** antagonist rPSGL-Ig. RESULTS: A concentration of 500 pM **LTC4** induced a threefold and sixfold elevation in leukocyte rolling flux and adhesion, respectively, compared to baseline values ($p < 0.05$). Administration of rPSGL-Ig had no effect on **LTC4**-induced leukocyte rolling flux but significantly attenuated the increase in the fraction of rolling leukocytes ($p < 0.05$). In addition, rPSGL-Ig inhibited the **LTC4** -induced reductions in leukocyte rolling velocity ($p < 0.001$). Finally, **LTC4** -induced leukocyte adhesion in animals pretreated with rPSGL-Ig was reduced by 60%, compared to vehicle-treated animals ($p < 0.05$). CONCLUSIONS: **LTC4** induces leukocyte rolling and adhesion in feline mesenteric venules in a dose-dependent manner. Administration of rPSGL-Ig inhibits **LTC4** -induced reductions in leukocyte rolling velocity and attenuates the elevation in the fraction of rolling leukocytes produced by **LTC4** stimulation. This suggests that rPSGL-Ig may be used to reduce leukocyte rolling and adhesion, and subsequently attenuate tissue injury during inflammation.

Record Date Created: 20010411

3/7/3 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10227427 99216124 PMID: 10198324

Assessment of the mechanism of juxtacrine activation and adhesion of leukocytes in liver microcirculation.

Carvalho-Tavares J; Fox-Robichaud A; Kubes P
Immunology Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

American journal of physiology (UNITED STATES) Apr 1999, 276 (4 Pt 1) pG828-34, ISSN 0002-9513 Journal Code: 0370511
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Leukotriene C4 (**LTC4**), histamine, and other mediators can induce expression of **P-selectin** and platelet-activating factor (PAF) on venular endothelium to recruit leukocytes in vivo and in vitro via a juxtacrine mechanism of adhesion. The objective of this study was to assess the effect of histamine and **LTC4** on the leukocyte recruitment in the liver and to study the components and molecular mechanisms involved in this process. We visualized the hepatic microvasculature using intravital microscopy and we determined that **LTC4** (20 nM) but not histamine (0.1, 0.3, or 1 mM) induced leukocyte recruitment in the liver microcirculation. Histamine could induce leukocyte recruitment but only in the presence of an antihistaminase. The **LTC4** -induced leukocyte

recruitment occurred primarily in sinusoids (not venules) and was not inhibitable by three different anti-**P-selectin** antibodies (5H1, RMP-1, and RB40). Leukocyte recruitment in **P-selectin**-deficient mice, intercellular adhesion molecule 1 (ICAM-1)-deficient mice, and mice treated with a PAF antagonist was of the same magnitude as in wild-type animals in response to **LTC4**. Although PAF alone could induce adhesion in both sinusoids and postsinusoidal venules, this chemotactic agent was not involved in **LTC4** -induced adhesion in the liver. Finally, an overlapping role for **P-selectin** and ICAM-1 was ruled out as **LTC4** induced leukocyte recruitment in **P-selectin** and ICAM-1 double-deficient mice. These data demonstrate that **LTC4** does not activate the known early mechanisms of leukocyte recruitment, including **P-selectin**, PAF, or ICAM-1 in the hepatic microvasculature.
Record Date Created: 19990518

3/7/4 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09587802 98026174 PMID: 9379052
Differential roles of selectins and the alpha4-integrin in acute, subacute, and chronic leukocyte recruitment in vivo.
Johnston B; Walter U M; Issekutz A C; Issekutz T B; Anderson D C; Kubes P
Immunology Research Group, University of Calgary, Alberta, Canada.
Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Nov 1 1997, 159 (9) p4514-23, ISSN 0022-1767 Journal Code: 2985117R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Adhesion blocking mAbs specific for rat P-, E-, and L-selectin and the alpha4-integrin were used to characterize leukocyte recruitment mechanisms in models of **LTC4** (acute), LPS (subacute), and adjuvant-induced (chronic) inflammation. Intravital microscopy was employed to measure leukocyte rolling and adhesion in rat mesenteric venules. Superfusing the mesentery with 20 nM **LTC4** elicited an increase in leukocyte rolling (66.8 +/- 3.8 vs 18.2 +/- 3.2 cells/min control) that was completely eliminated by an anti-rat **P-selectin** mAb. Superfusion with 1 microg/ml LPS induced a significant increase in leukocyte rolling within 15 min (73 +/- 8 vs 33 +/- 6 cells/min control). Rolling increased further starting at 105 min and peaked by 150 min (141 +/- 23 cells/min). LPS-induced leukocyte rolling was eliminated during the first 90 min by the **P-selectin** mAb. The later increase in leukocyte rolling was not prevented by a second treatment with **P-selectin** mAb or a function-blocking mAb against rat E-selectin. This later phase of leukocyte rolling was blocked by treatments with mAbs against either the alpha4-integrin or L-selectin. Twelve days following Mycobacterium butyricum immunization, 300 to 500 rolling cells/min were observed. This could be reduced approximately 50 to 60% by mAb against either the alpha4-integrin or L-selectin. The combination of both mAbs eliminated approximately 90% of rolling. Neither the P- nor E-selectin mAbs reduced rolling in this chronic inflammatory model. This study highlights differences in leukocyte adhesive mechanisms elicited by different stimuli and at different time points within the same vascular bed.
Record Date Created: 19971112

3/7/5 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09385202 97296901 PMID: 9152370
Cysteinyl leukotrienes induce **P-selectin** expression in human endothelial cells via a non-CysLT1 receptor-mediated mechanism.
Pedersen K E; Bochner B S; Undem B J

Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Journal of pharmacology and experimental therapeutics (UNITED STATES) May 1997, 281 (2) p655-62, ISSN 0022-3565 Journal Code: 0376362
Contract/Grant No.: HL-38095; HL; NHLBI; HL-49545; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Cysteinyl leukotrienes are bioactive lipid mediators known to possess potent proinflammatory actions. Included in these are effects on vascular endothelium to promote surface expression of the adhesion molecule **P-selectin** . In the present study we were interested in investigating the receptor mechanism(s) involved in cysteinyl leukotriene-induced endothelial **P-selectin** expression. As such we examined the effect of several potent and selective cysteinyl leukotriene receptor antagonists on this response. Incubation of cultured human umbilical vein endothelial cells (HUVEC) with the cysteinyl leukotrienes leukotriene C4 (**LTC4**) or leukotriene D4 (LTD4) induced surface expression of **P-selectin** which was concentration dependent and rapid in onset. Expression of endothelial **P-selectin** induced by either **LTC4** or LTD4 was not blocked however by pretreatment of HUVEC with the selective cysteinyl leukotriene-1 (CysLT1) receptor antagonists SKF 104353, pranlukast or zafirlukast before agonist exposure. In contrast, SKF 104353 effectively antagonized the **LTC4** -induced contractions in isolated human bronchial smooth muscle preparations, shifting the agonist dose-response curve to the right by some 3 log-fold in this tissue. The present results suggest that cysteinyl leukotrienes induce surface expression of endothelial **P-selectin** via a mechanism independent of the CysLT1 receptor.

Record Date Created: 19970612

3/7/6 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08955894 96310965 PMID: 8690917

Lipoxin A4 and B4 inhibit leukotriene-stimulated interactions of human neutrophils and endothelial cells.

Papayianni A; Serhan C N; Brady H R
Department of Medicine, Brockton-West Roxbury Department of Veterans Affairs Medical Center, Boston, MA 02132, USA.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Mar 15 1996, 156 (6) p2264-72, ISSN 0022-1767 Journal Code: 2985117R
Contract/Grant No.: DK 44380; DK; NIDDK; GM 38765; GM; NIGMS; P01-DK 50305; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Lipoxins are bioactive eicosanoids that are generated within the vascular lumen by leukocytes and transcellular biosynthetic routes during multicellular responses. Polymorphomuclear neutrophils (PMN) and endothelial cells express high affinity receptors for lipoxins, engagement of which invokes profiles of signaling events that differ from other lipid mediators. In this work, we report that lipoxins are potent inhibitors of PMN-endothelial cell interactions triggered by leukotrienes via dual-pronged actions with PMN and endothelial cells. Both lipoxin A4(LXA4) and B4(LXB4) blocked PMN migration stimulated by leukotriene B4 (LTB4), a well established agonist for PMN recruitment, a transmigration assay in vitro. Lipoxins were almost as effective in this regard as the pharmacologic LTB4 receptor antagonist, ONO 4057, and the blocking anti-CD18 mAb, R15.7. LXA4 and LXB4 blunted PMN transmigration, in part by inhibiting beta 2 integrin-dependent PMN adhesion. These modulatory actions

of lipoxins were evident at subnanomolar concentrations, rapid in onset, and attenuated by prior exposure of PMN to a tyrosine kinase inhibitor, genistein.The peptidoleukotrienes, leukotriene C4 (**LTC4**) and leukotriene D4 (LTD4) also provoked PMN-endothelial cell adhesion, but via a different mechanism than LTB4. Both **LTC4** and LTD4 enhanced endothelial adhesiveness for PMN, in part, by stimulating mobilization of **P-selectin** from intracellular Weibel-Palade bodies. LXA4 and LXB4, but not other lipoxygenase products, blocked **P-selectin** mobilization induced by peptidoleukotrienes and attenuated **P-selectin** -mediated PMN-endothelial cell adhesion. These results indicate that lipoxins attenuate PMN-endothelial cell interactions supported by selectins and beta 2 integrins in vitro, and are potential endogenous lipid-derived modulators of PMN trafficking in host defense, inflammation, and other vascular events.
Record Date Created: 19960826

3/7/7 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08715875 96080086 PMID: 7586318
Peptido-leukotrienes are potent agonists of von Willebrand factor secretion and **P-selectin** surface expression in human umbilical vein endothelial cells.
Datta Y H; Romano M; Jacobson B C; Golan D E; Serhan C N; Ewenstein B M
Hematology-Oncology Division, Brigham and Women's Hospital, Boston, Mass., USA.
Circulation (UNITED STATES) Dec 1 1995, 92 (11) p3304-11, ISSN 0009-7322 Journal Code: 0147763
Contract/Grant No.: HL-15157; HL; NHLBI; HL-32854; HL; NHLBI; HL-45629; HL; NHLBI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: The peptido-leukotrienes (LTs) and lipoxins (LX) are produced by platelets through the transcellular conversion of leukocyte-derived LTA4 at sites of vascular inflammation and injury, such as during coronary artery balloon angioplasty. We studied the actions of these eicosanoids on vascular endothelium. METHODS AND RESULTS: We found that stimulation of cultured human umbilical vein endothelial cells (EC) with **LTC4** and LTD4 resulted in the release of high-molecular-weight multimers of von Willebrand factor (vWF) in a concentration- and time-dependent fashion, as measured by ELISA. Neither LXA4 nor LXB4 stimulated vWF release. **LTC4** and LTD4 also stimulated a rapid increase in the surface expression of **P-selectin** indicated by increased binding of anti-**P-selectin** monoclonal antibody-coated beads. Fluorescence cytometry detected prolonged peaks of [Ca2+]i in EC in response to concentrations of thrombin and LTD4 that induce near-maximal vWF secretion. In contrast, concentrations of **LTC4** that induce similar levels of vWF secretion produced only asynchronous oscillations of [Ca2+]i in most EC and rarely induced prolonged peaks of [Ca2+]i. Depletion of external Ca2+ had no apparent impact on LT-stimulated [Ca2+]i transients and vWF secretion, implicating an intracellular pool as the source of this response. Staurosporine, sphingosine, and H-7 each had only modest effects on peptido-LT-induced vWF secretion, suggesting that protein kinase C is not a primary mediator of peptido-LT-induced exocytosis. Inhibitors of cyclooxygenase and platelet-activating factor had no effect on peptido-LT-mediated vWF secretion. CONCLUSIONS: Through the induction of vWF secretion and **P-selectin** surface expression, peptido-LTs are likely to play an important role in the interrelated processes of hemostasis and inflammation.
Record Date Created: 19951228

3/7/8 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08655760 96027696 PMID: 7554141
Leukotriene C4/D4 induces **P-selectin** and sialyl Lewis(x)-dependent alterations in leukocyte kinetics in vivo.
Kanwar S; Johnston B; Kubes P
Immunology Research Group, University of Calgary, Canada.
Circulation research (UNITED STATES) Nov 1995, 77 (5) p879-87, ISSN 0009-7330 Journal Code: 0047103
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The objective of this study was to assess the effect of leukotriene C4 (**LTC4**) on the flux of rolling leukocytes, leukocyte rolling velocity, and leukocyte adhesion in postcapillary venules in vivo and to study the underlying molecular mechanisms involved. **LTC4** (20 nmol/L) induced a rapid and significant increase in leukocyte rolling flux that was inhibitable by an anti-**P-selectin** antibody and soluble sialyl Lewis(x) (sLe(x)). **LTC4** also induced a significant reduction in leukocyte rolling velocity, an event that was independent of **P-selectin** but entirely dependent on sLe(x). This **LTC4**-induced reduction in leukocyte rolling velocity was independent of any hemodynamic alterations. Another **P-selectin** effector, histamine, did not affect leukocyte rolling velocity even at > 5000 times the concentration of **LTC4**. Treatment with an anti-L-selectin antibody had no effect on the **LTC4** -induced increase in leukocyte rolling or reduction in rolling velocity. Inhibition of **LTC4** bioconversion to LTD4 by pretreatment with L-serine (100 mumol/L) prevented the **LTC4**-induced increase in leukocyte rolling flux and the **LTC4**-induced reduction in leukocyte rolling velocity. A subtle, yet significant, increase in leukocyte adhesion was also observed with **LTC4**. Pretreatment with a platelet-activating factor receptor antagonist returned the **LTC4** -induced leukocyte rolling velocity to baseline levels. The addition of a very low concentration of platelet-activating factor (1 nmol/L) induced significant leukocyte adhesion in the presence of **LTC4** but not histamine.(ABSTRACT TRUNCATED AT 250 WORDS)
Record Date Created: 19951120

3/7/9 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08401456 95159084 PMID: 7531878
Polymorphonuclear leukocyte-platelet interaction: role of P-selection in thromboxane B2 and leukotriene C4 cooperative synthesis.
Maugeri N; Evangelista V; Celardo A; Dell'Elba G; Martelli N; Piccardoni P; de Gaetano G; Cerletti C
Giulio Bizzozero Laboratory of Platelet and Leukocyte Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy.
Thrombosis and haemostasis (GERMANY) Sep 1994, 72 (3) p450-6, ISSN 0340-6245 Journal Code: 7608063
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In PMN/platelet suspensions stimulated by fMLP giant mixed aggregates are formed and TxB2 and **LTC4** are synthesized as the result of the cooperation in the arachidonic acid (AA) metabolism during cell/cell contact. PMN-derived cathepsin G induced the expression of **P-selectin** on platelet surface. GE12, an antibody against **P-selectin** , significantly reduced mixed cell aggregates. GE12 did not

affect platelet aggregation induced by PMN-derived supernatants, indicating that the inhibitory effect of GE12 on mixed cell aggregation depends on inhibition of PMN/platelet adhesion. GE12 significantly reduced TxB2 and **LTC4** production in PMN/platelet mixed cell suspensions stimulated by fMLP. As previously reported, synthesis of 3H-TxB2 in 3H-AA-labeled PMN/unlabeled platelets indicates that platelets utilize 3H-AA from PMN. 3H-**LTC4** production in unlabeled PMN/3H-AA-labeled platelets indicates that bidirectional routes are utilized in this system for **LTC4** synthesis. GE12 significantly reduced 3H-TxB2 and 3H-**LTC4** synthesis. These results show that cathepsin G released by activated PMN induces the expression of **P-selectin** on platelet membrane: this adhesive glycoprotein modulates cell-cell contact and transcellular metabolism of AA.

Record Date Created: 19950314

3/7/10 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08355397 95116480 PMID: 7816774

The present knowledge of the inflammatory process and the inflammatory mediators.

Leirisalo-Repo M
Second Department of Medicine, University of Helsinki, Finland.

Pharmacology & toxicology (DENMARK) 1994, 75 Suppl 2 p1-3, ISSN 0901-9928 Journal Code: 8702180
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Endothelial damage, synovial oedema, fibrin deposition, polymorphonuclear cell (PMN) invasion, and mild lining cell hyperplasia characterize acute inflammatory arthritis. Later on, perivascular tissue is infiltrated by mononuclear cells. The early events are mediated by interactions between PMNs and endothelial cells. Both parts in the adhesion event are activated with multiple stimuli resulting in complex interactions of varying intensity and duration. Adhesion molecules present on the surface of PMNs (L-selectin) or induced by inflammatory stimuli (beta 2-integrins) mediate PMN adhesion to activated endothelium, which has counter receptors (E-selectin for L-selectin and ICAM-1 and ICAM-2 for beta 2-integrins). At the initial phase L-selectin initiates the rolling of PMNs on endothelial cells. Further stimuli result in a more prolonged adhesion between PMNs and endothelium. At the side of endothelium, induction of **P-selectin** and PAF by histamine, thrombin and **LTC4** contribute to the acute rolling of PMNs on endothelial surface. Tumor necrosis factor (TNF), interleukin-1 (IL-1) and lipopolysaccharide activate endothelial cells to synthesize interleukin-8 (IL-8), a potent chemotactic and proadhesive mediator for PMNs, and further adhesion molecule (E-selectin), a mediator of long-term adhesion between PMN and endothelium. After adhesion and migration to the focus of inflammation, PMNs induce inflammation by aggregating, releasing hydrolyzing enzymes, generating lipid peroxidation products such as prostaglandins and LTB4, and oxygen derived free radicals. In studies on the pathogenesis of seronegative spondyloarthropathies, we have shown persistently aberrant PMN function evidenced by enhanced chemotaxis and high production of toxic oxygen derived free radicals by PMN.(ABSTRACT TRUNCATED AT 250 WORDS) (12 Refs.)

Record Date Created: 19950206

3/7/11 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

123283576 CA: 123(21)283576m JOURNAL

Leukotriene C4/D4 induces P-selectin and sialyl Lewisx-dependent alterations in leukocyte kinetics in vivo
AUTHOR(S): Kanwar, Samina; Johnston, Brent; Kubes, Paul
LOCATION: Immunology Research Group, Univ. of Calgary, Calgary, Can.,
JOURNAL: Circ. Res. DATE: 1995 VOLUME: 77 NUMBER: 5 PAGES: 879-87
CODEN: CIRUAL ISSN: 0009-7330 LANGUAGE: English
SECTION:
CA215010 Immunochemistry
CA202XXX Mammalian Hormones
CA214XXX Mammalian Pathological Biochemistry
IDENTIFIERS: LTC4 P selectin leukocyte rolling, sialyl Lewis x leukocyte adhesion LTD4
DESCRIPTORS:
Leukocyte...
LTC4 induces P-selectin- and sialyl Lewis-dependent rolling in post-capillary venules by
Vein,venule...
LTC4 induces P-selectin- and sialyl Lewis-dependent rolling of leukocytes on
Adhesion,bio-... Blood-group substances,Lex, sialyl...
Receptors,P-selectins...
LTC4 induces P-selectin- and sialyl Lewis-dependent rolling of leukocytes on post-capillary venules
Immunity,cell-mediated... Inflammation...
LTC4 induces P-selectin- and sialyl Lewisx-dependent leukocyte rolling in post-capillary venules
CAS REGISTRY NUMBERS:
65154-06-5 in LTC4 induction of P-selectin- and sialyl Lewis-dependent rolling of leukocytes on post-capillary venules
73836-78-9 LTC4 induces P-selectin- and sialyl Lewis-dependent rolling of leukocytes on post-capillary venules on conversion to
72025-60-6 P-selectin- and sialyl Lewisx-dependent induction of leukocyte rolling in post-capillary venules by

3/7/12 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

122029779 CA: 122(3)29779j JOURNAL
Polymorphonuclear leukocyte-platelet interaction: role of P-selectin in thromboxane B2 and leukotriene C4 cooperative synthesis
AUTHOR(S): Maugeri, Norma; Evangelista, Virgillio; Celardo, Antonio; Dell'Eba, Giuseppe; Martelli, Nicola; Piccardoni, Paola; de Gaetano, Giovanni; Cerletti, Chiara
LOCATION: Giulio Bizzozero Lab. Platelet Leukocyte Pharmacol., Ist. Ricerche Farmacol. Mario Negri, Santa Maria Imbaro, Italy
JOURNAL: Thromb. Haemostasis DATE: 1994 VOLUME: 72 NUMBER: 3 PAGES: 450-6 CODEN: THHADQ ISSN: 0340-6245 LANGUAGE: English
SECTION:
CA215010 Immunochemistry
CA202XXX Mammalian Hormones
IDENTIFIERS: polymorphonuclear leukocyte platelet adhesion P selectin, TXB2 LTC4 P selectin leukocyte platelet
DESCRIPTORS:
Adhesion,bio-... Blood platelet... Leukocyte,polymorphonuclear...
Receptors,P-selectins...
role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.
CAS REGISTRY NUMBERS:
506-32-1 arachidonic acid metab. modulated by cathepsin G-induced expression of P-selectin on platelet surface
56645-49-9 polymorphonuclear leukocyte-derived cathepsin G induces the expression of P-selectin on platelet surface

54397-85-2 72025-60-6 role of P-selectin-mediated polymorphonuclear leukocyte-platelet adhesion in TXB2 and LTC4 prodn. and metab.
?

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L1: Entry 8 of 40 File: USPT Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6299897 B1
TITLE: Inhibition of selectin binding

Brief Summary Text (6):
Amongst the plurality of adhesion molecules that have been described, three have been collected together in a category known as selecting. One was formerly known as ELAM-1, and was identified using inhibitory monoclonal antibodies against cytokine-activated endothelial cells. Another was formerly designated as PADGEM, GMP-140, or CD61. It was originally identified on platelets, and is now known as P-selectin. A third identified on lymphocytes was formerly designated as mLHR, Leu8, TQ-1, gp90.sup.MEL, Lam-1, or Lecam-1, and is now known as L-selectin. The selectins were grouped together on the basis of a structural similarity, before very much was known about their binding specificity. All are single chain polypeptides having a carbohydrate binding domain near the N-terminus, an EGF repeat, and anywhere between 2 to 9 modules of .about.60 amino acids each sharing homology with complement binding proteins. For general reviews, the reader is referred to Lasky (Annu. Rev. Biochem. 64:113, 1995) and Kansas (Blood 88:3259, 1996).

Brief Summary Text (12):
P-selectin is a transmembrane glycoprotein of .about.140 kDa, substantially larger than E-selectin. It was originally described on platelets, in which it may be found in I- and dense-granules. Upon activation of platelets with a mediator like thrombin, P-selectin is rapidly redistributed to the cell surface. In endothelial cells, it is found in granules known as Weibel-Palade bodies, from which it is redistributed to the surface upon activation with histamine. Shuttling of P-selectin to storage granules appears to be mediated by a sorting signal present in the cytoplasmic domain, and apparently unique in comparison with E-selectin.

Brief Summary Text (13):
Accordingly, P-selectin differs from E-selectin in that it may be rapidly expressed from storage granules rather than requiring de novo synthesis. P-selectin binds carbohydrate ligands present on neutrophils, monocytes, and memory T cells. Not only is P-selectin in a preformed state, its expression is stimulated by mediators such as histamine which in turn are preformed and stored in the granules of inflammatory cells. The adherence of leukocytes to P-selectin rather than E-selectin on endothelial cells is perhaps the initial event that occurs for recruitment of these cells to an injured site. Interference with P-selectin binding may be particularly important when it is desirable to limit leukocyte migration.

Brief Summary Text (14):
The presence of P-selectin on platelets suggests additional unique biological roles compared with the other selecting. In one hypothesis, sites of tissue injury may be acutely enriched with short-acting platelet activators, and active platelets expressing P-selectin may directly recruit other leukocytes. In another hypothesis, neutrophils or monocytes at an inflamed site may be able to catch platelets by way of the P-selectin, which in turn could lead to clot formation or additional mediator release. In an experimental thrombus model, it has been observed that platelets accumulate first at the injury site, followed by leukocyte adherence and fibrin deposition. Both of the latter two steps was inhibited by antibodies against P-selectin (Palabrica et al., Nature 359:848, 1992).

Brief Summary Text (18):
Each of the selectins shows a fine specificity in terms of the carbohydrate

requirement for binding. All three selectins bind sialylated fucooligosaccharides, of which the prototype is the tetrasaccharide sialyl Lewis.sup.x (sLe.sup.x). Direct binding experiments between synthetic carbohydrates and isolated selectins has permitted a more detailed dissection of the binding requirements (e.g., Brandley et al., Glycobiology 3: 633, 1993). E- and L-selectin require an I2-3 linkage for the sialic acid in sLe.sup.x, whereas P-selectin can recognize sialic acid in an I2-6 linkage. P-selectin also does not require a hydroxyl group in the fucose 2- and 4-positions. P- and L-selectin bind sulfated structures like sulpho-Le.sup.x -(Glc)-cer and sulfatides in a manner largely independent of divalent cations, whereas E-selectin binding is exquisitely sensitive to the presence of cations. Binding of P- and L-selectin to sulfated carbohydrates is only inhibitible by other sulfated carbohydrates, whereas E-selectin does not have this requirement.

Brief Summary Text (20):
Each of the selectins has a different family of natural ligands on the surface of the opposing cell (see McEver et al., 270:11025, 1995). E-selectin binds strongly to a ligand designated ESL-1. In contrast, antibody blocking studies indicate that essentially all the binding sites for P-selectin on leukocytes are attributable to an O-glycosylated protein designated PSGL-1 (P-selectin glycoprotein ligand 1) (Moore et al., J. Cell Biol. 128:661, 1995). The natural ligands identified for L-selectin is neither of these, but include other glycoproteins with the designations GlyCAM-1, CD34, and MAdCAM-1.

Brief Summary Text (21):
The binding specificity indicates that at least two of the three selectins must be recognizing a ligand component beyond the sLe.sup.x structure. In addition to the oligosaccharide, P-selectin must bind a site on PSGL-1 with features different from ESL-1 and from other mucin-like O-glycosylated proteins, such as CD43.

Brief Summary Text (24):
The sulfate component has been mapped more precisely in the structure of the P-selectin ligand PSGL-1. The requirement in P-selectin is provided by one or more sulfated tyrosines near the N-terminus of the polypeptide backbone, separate from the glycosylation site.

Brief Summary Text (25):
Wilkins et al. (J. Biol. Chem. 270:22677, 1995) demonstrated that PSGL-1 synthesized in human HL-60 cells can be metabolically labeled with [.sup.35 S]sulfate. It was shown that most of the .sup.35 S label was incorporated into the polypeptide in the form of tyrosine sulfate. Treatment of PSGL-1 with a bacterial arylsulfatase released sulfate from tyrosine, and resulted in a concordant decrease in binding to P-selectin.

Brief Summary Text (26):
Pouyani et al. (Cell 83:333, 1995) demonstrated that selective inhibitors of sulfation compromised binding of HL-60 cells to soluble P-selectin but not E-selectin. The cell-surface expression of sLe.sup.x or the polypeptide were not compromised by treatment. Deletion analysis of isolated PSGL-1 constructs localized the binding component to residues 20-40. The segment contains three tyrosine residues, and when these were changed to phenylalanine, P-selectin binding activity was abolished. Furthermore, when the 20 amino acid segment was fused on to a different protein, it was again sulfated during biosynthesis and had binding activity for P-selectin. These authors suggested that the sulfated tyrosines interact with P-selectin domain, which is located closer in the protein sequence to the membrane spanning domain.

Brief Summary Text (27):
Sako et al. (Cell 83:323, 1995) performed another series of binding experiments using the extracellular domain of PSGL-1 expressed as a fusion protein. The assay required fucosylation of the protein and cations in the assay medium, consistent with a dependence on carbohydrates like sLe.sup.x. Mutation of the putative N-linked glycosylation sites had no effect on selectin binding, suggesting that the carbohydrate requirement was O-linked. However, mutation of three tyrosines to phenylalanine abrogated binding activity for P-selectin. Binding of E-selectin, for which PSGL-1 can also act as a ligand, was not affected by removal of the sulfation

sites.

Brief Summary Text (28):
The binding affinity of P- and L-selectin for sLe.sup.x is in the mM range (Nelson et al., J. Clin Invest. 91:1157,1993). In contrast, the affinity of P-selectin for the natural ligand is in the nM range (Moore et al., J. Cell Biol. 112:491, 1991), a difference in potency of .about.10.sup.6 fold. Synthetic oligosaccharides containing multiple sLe.sup.x units only partly make up the difference, so the effect is not just due to ligand valency. The disparity is also attributable to the requirement of P- and L-selectin for a strong anionic determinant, like the sulfotyrosines on PSGL-1. Compounds effective in the same concentration range as PSGL-1 must be able to supply a similarly effective determinant combination.

Brief Summary Text (39):
Also embodied are compositions for treating a disease characterized by local alteration in the adherence of leukocytes or cancer cells to vascular endothelium, platelets or lymphatic tissue, comprising a polymerized lipid composition comprising a sheet of lipids wherein a proportion of the lipids are covalently crosslinked, a proportion of the lipids have an attached saccharide, and a proportion of the lipids not having an attached saccharide have an acid group that is negatively charged at neutral pH. Diseases of interest include but are not limited to cardiac disease (such as ischemia reperfusion injury, myocardial infarction, myocarditis, restenosis, and deep vein thrombosis), hemmorhagic shock, arthritis, asthma, and metastatic cancer.

Drawing Description Text (2):
FIG. 1 is a drawing of two polymerized glycoliposomes showing an expanded detail of the chemical structure. Structure "A" is able to inhibit the binding of P-selectin to HL-60 cells at an oligosaccharide concentration below 2 nM, while Structure "B" has essentially no activity. The vesicles are unilamellar and made up of single-chain lipids with diyne groups cross-linked using UV light. Conjugated to about 5% of the lipids are analogs of the sLe.sup.x oligosaccharide. The preparations differ in terms of the outward facing determinants displayed by the neighboring lipids. In structure "A", the neighboring lipids provide carboxylic acid groups, which have a negative charge at neutral pH. In structure "B", the neighboring lipids are neutral. The negatively charged lipids work synergistically with the sLe.sup.x analog to supply P-selectin binding activity, just as sulfotyrosine works synergistically with sLe.sup.x in the natural ligand. P- and L-selectin differ from E-selectin in the requirement for a negative charge determinant in binding.

Drawing Description Text (3):
FIGS. 2A and 2B depict some of the aspects of selectin binding. In FIG. 2A the boxed panel shows the receptor ligand pairs known for L-, P- and E-selectin. They are depicted on the same cell for convenience, but participate in different ways to cell adhesion and migration. FIG. 2B is a detail showing the dual binding site model for P-selectin. In the ligand PSGL-1, the negative groups correspond to three sulfotyrosine residues. In contrast, there is no evidence for a separate anion binding site for E-selectin.

Drawing Description Text (5):
FIG. 4 is a titration curve for the inhibition of P-selectin binding to HL-60 cells by glycoliposomes. In order of decreasing potency (left to right) the compositions are comprised of sLe.sup.x analog plus acidic lipids; lactose plus acidic lipids; maltose plus acidic lipids; and sLe.sup.x analog plus neutral lipids.

Detailed Description Text (12):
FIG. 1 shows exemplary lipid compositions of this invention, in which an analog of sLe.sup.x is displayed on the surface of a polymerized unilamellar liposome. Only the first structure demonstrated inhibitory activity for P-selectin binding in the bioassay, underlining the importance of the anionic component in the composition.

Detailed Description Text (69):
Inhibitors that are selective for P- and/or L-selectin are of particular interest, because of recent observations that E-selectin antagonists can lead to conditions

reminiscent of leukocyte adhesion deficiency disease (LAD-2), where neutrophils do not adhere normally to endothelial tissues, and recurrent bacterial infections of the lung, skin, and gingival tissues are common. Example 3 provides illustrations of selective polymerized liposomes. Non-sulfated sugars like sLe.sup.x and the neutral disaccharides lactose and maltose are selective for L- and P-selectin when presented in the context of carboxy-terminated lipids. sLe.sup.x is also selective in the context of hydroxyl-terminated lipids. Liposomes with sulfate groups either on sulfo Le.sup.x or on a lipid in combination with sLe.sup.x were not selective.

Detailed Description Text (80):
A convenient one-cell assay for P-selectin inhibitors makes use of HL-60 cells, available from the ATCC. HL-60 cells naturally express the PSGL-1 antigen at about 36,000 sites per cell (Ushiyama et al., J. Biol. Chem. 268:15229, 1993). The assay is described in Brandley et al. (Glycobiol. 3:633, 1993). Briefly, an E or P-selectin chimera is incubated with biotinylated goat F(ab'), anti-human IgG Fc, and an alkaline phosphatase-streptavidin conjugate for 30 min. This complex is then incubated with potential inhibitors for .about.45 min at 37.degree. C. 50 TL of the mixture is added to each well of round-bottom microtiter plates previously blocked with BSA. An equal volume of an HL-60 cell suspension is added and the plate is incubated for 45 min at 4.degree. C. Cells are pelleted to the well bottoms by centrifugation, washed, and developing using p-nitrophenyl phosphate.

Detailed Description Text (81):
Other one-cell assays are done with cell isolates rather than cell lines. The ability to inhibit neutrophil adhesion to purified P-selectin immobilized on plastic wells can be determined using the assay described by Geng, et al. (Nature 343:757, 1990). Briefly, human neutrophils are isolated from heparinized whole blood by density gradient centrifugation on Mono-Poly.TM. resolving media (Flow Laboratories), and suspended in Hanks' balanced salt solution containing Ca.sup.++, Mg.sup.++, and human serum albumin (HBSS/HSA). P-selectin is obtained by recombinant expression or isolated from outdated human platelet lysates by immunoaffinity chromatography on antibody S12-Sepharose.TM. and ion-exchange chromatography on a Mono-Q.TM. column (U.S. Pat. No. 5,464,935). The P-selectin is coated onto microtiter plate wells at 5 Tg/mL. Cells are added at .about.2.times.10.sup.5 per well, incubated at 22.degree. C. for 20 min. The wells are then filled with HBSS/HSA, sealed with acetate tape, and centrifuged. After discarding nonadherent cells and supernates, the contents of each well are solubilized with 0.5% hexadecyltrimethylammonium bromide in phosphate buffer and assayed for myeloperoxidase activity (Ley et al., Blood 73:1324, 1989).

Detailed Description Text (96):
Since the selectins have several functions related to leukocyte adherence, inflammation, and coagulation, compounds that interfere with binding of P-selectin or L-selectin can be used to modulate the pathological consequences of these events.

Detailed Description Text (98):
Reperfusion injury is a major problem in clinical cardiology. Therapeutic agents that reduce leukocyte adherence in ischemic myocardium can significantly enhance the therapeutic efficacy of thrombolytic agents. Thrombolytic therapy with agents such as tissue plasminogen activator or streptokinase can relieve coronary artery obstruction in many patients with severe myocardial ischemia prior to irreversible myocardial cell death. However, many such patients still suffer myocardial necrosis despite restoration of blood flow. Reperfusion injury is known to be associated with adherence of leukocytes to vascular endothelium in the ischemic zone, presumably in part because of activation of platelets and endothelium by thrombin and cytokines that makes them adhesive for leukocytes (Romson et al., Circulation 67:1016, 1983). The adherent leukocytes can migrate through the endothelium and destroy ischemic myocardium just as it is being rescued by restoration of blood flow. Ischemia may occur pursuant to a myocardial infarction or as a result of complications of surgery, such as deep vein thrombosis. Another inflammatory condition of concern in cardiology is restenosis.

Detailed Description Text (101):
Tumor cells from many malignancies (including carcinomas, lymphomas, and sarcomas)

metastasize to distant sites through the vasculature. The mechanisms for adhesion of tumor cells to endothelium and their subsequent migration are not well understood, but may be similar to those of leukocytes in at least some cases. The association of platelets with metastasizing tumor cells has been well described, suggesting a role for platelets in the spread of some cancers. It has been reported that P-selectin binds to tumor cells in human carcinoma tissue sections and cell lines derived from carcinomas (Aruggo et al., Proc. Natl. Acad. Sci. USA 89:2292, 1992). In addition, certain tumors may themselves express selectins or selectin ligands, which may participate in the adherence of metastasizing cells to endothelial cells or HEV at a new site.

Detailed Description Text (102):
Antagonists of P-selectin may be beneficial for blocking platelet-leukocyte interaction as thrombi develop (Welpy et al., Biochim. Biophys. Acta 117:215, 1994). In baboons, administration of anti P-selectin decreased fibrin deposition into Dacron graft implants without diminishing platelet accumulation into the grafts (Palabrica et al., Nature 359, 848, 1992). The results suggest that the trapping of leukocytes, via interaction with platelets, may contribute to the deposition of fibrin. Blocking P-selectin should prevent this interaction and may have value as an anti-thrombogenic therapy.

Detailed Description Text (114):
1. Inhibiting leukocyte adhesion or migration, comprising administering a P-selectin inhibitor so as to inhibit binding between a vascular endothelial cell and a leukocyte selected from the group consisting of neutrophils, monocytes, eosinophils, and lymphocytes bearing a P-selectin ligand, thought to be memory T cells. The inhibiting can be performed either by introducing the inhibitor into an environment where the interacting cells come into contact, particularly near the affected site, or contacting the cell bearing the selectin with the inhibitor in the absence of the cell bearing the ligand.

Detailed Description Text (115):
2. Inhibiting platelet aggregation or fibrin deposition by administering a P-selectin inhibitor to an environment containing platelets or susceptible of accumulating platelets.

Detailed Description Text (140):
Ability of the compositions prepared in Example 1 to inhibit selectin binding was tested in a standard bioassay. The assay for measuring P-selectin binding to HL-60 cells was taken from the description in Brandley et al. (Glycobiol. 3:633, 1993). Briefly, P-selectin chimera is allowed to form a complex with biotinylated goat F(ab') anti-human IgG Fc and alkaline phosphatase-streptavidin, and is preincubated with inhibitors before mixing with HL-60 cells. The cells were pelleted by centrifugation and washed with TBS. Chromagen was added and the color that developed was read as an OD at 405 nm. All assays were run in quadruplicate.

Detailed Description Text (141):
FIG. 4 shows the inhibition titration curve for various polymerized glycoliposome preparations containing 5% carbohydrate-linked lipid. Open triangles: sLe.sup.x analog conjugate plus acidic lipids. Open circles: sLe.sup.x analog conjugate plus neutral lipids. Closed circles: lactose conjugate plus acidic lipids. Squares: maltose conjugate plus acidic lipids. It is evident from the results of this assay that the presence of the acidic lipid is critical for measurable inhibition, even when the most effective carbohydrate conjugate of those tested, the sLe.sup.x analog, is used. The neutral disaccharides lactose and maltose also have selectin inhibition activity when used alongside acidic lipids. All the compositions having a saccharide and a negatively charged lipid inhibited P-selectin binding in a dose-dependent fashion.

Detailed Description Text (146):
The possibility of intercalation of the liposomes into the cells, thereby effecting their ability to bind P-selectin, was also addressed. The cells were pretreated with the liposomes and washed to remove the liposomes prior to the addition of the P-selectin chimera. This did not result in any reduction in selectin binding to the cells. The inhibition was unaffected in experiments where the reagents and

inhibitors were added simultaneously to the microtiter plates.

Detailed Description Text (159):
The results support the following conclusions. First, the sulfated carbohydrate sulfo Le.sup.x analog has a very low IC.sub.50 (high inhibitory capacity) for L-, E- or P-selectin in a context of acidic or polar lipids (but not positively charged lipids). Where the saccharide is the non-sulfated sLe.sup.x analog, an acidic neighboring lipid is required for full inhibitory activity, which is selective for L- and P-selectin. Sulfate lipids support sLe.sup.x binding better than carboxylate lipids, even at a relative proportion of 50%. As in the preceding example, the presence of acid lipids turn ineffective neutral disaccharides like lactose and maltose into effective inhibitors. This effect occurred only for L- and P-selectin, since none of the neutral disaccharide compositions inhibited E-selectin binding. The contributory effect of acid groups to the binding of L- and P-selectin is consistent with the working hypothesis that the lipid acid groups fulfill a selectin binding requirement equivalent to what is provided by sulfotyrosine or its equivalent in the biological ligands.

Detailed Description Text (163):
Glycoliposomes containing 5% sulfo Le.sup.x analog and 95% hydroxyl-terminated lipid were tested in a flow adhesion assay (Alon et al., Nature 374:539, 1995). Briefly, P-selectin chimera is immobilized in a flow chamber and the affinity of HL-60 cells for this substrate is manifest for their ability to roll slowly along on the surface. The interaction is specific for the PSGL-1 mucin domain on the HL-60 cells and the inhibitor's ability to block cell adhesion under physiological flow rather than under static conditions. At a glycolipid concentration of 1 TM, this glycoliposome formulation was able to completely inhibit HL-60 cell rolling on P-selectin surfaces. The control liposome (without the carbohydrate) had no effect.

Detailed Description Text (180):
Treated animals in the cited studies responded to 400 Tg/kg of sLe.sup.x presented as a phospholipid liposome, or 1 mg/kg of the anti-L-selectin monoclonal antibody DREG-200. In the present experiment, polymerized liposomes are tested in a range of about 10-400 Tg of carbohydrate equivalent per kg body weight. An equal number of polymerized liposomes made of 100% neutral lipids is given at an equal dose (on a per-liposome basis) as vehicle control. To the extent that necrosis induced by other types of acute cardiac inflammatory events, such as myocarditis, restenosis and deep vein thrombosis, are mediated by similar mechanisms, the effective doses established in the cardiac reperfusion model may also be considered for these conditions.

[redacted]

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APPLICANTS

MICHAEL J. EPPIHIMER, ANDOVER, MA ;
ROBERT G. SCHAUB, ANDOVER, MA ;
ALAN HARRIS, CAMBRIDGE, MA ;

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Verified and Acknowledged	Examiner's Signature Initials				

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PATENT & TRADEMARK DEPARTMENT 2B
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ONE CAMPUS DRIVE
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TITLE

INHIBITION OF THROMBOSIS BY TREATMENT WITH DIMERIC PSGL-1

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SUBMISSION OF PROVISIONAL PATENT APPLICATION

Pursuant to 35 USC 111(b) and 37 CFR 1.51(c) the applicants named herein present the following to establish a Provisional Patent Application.

Applicants/Inventors: Michael J. Eppihimer, Andover, Massachusetts; Robert G. Schaub, Andover, Massachusetts; and Alan Harris, Cambridge, Massachusetts.

Title: INHIBITION OF THROMBOSIS BY TREATMENT WITH DIMERIC PSGL-1

Attorney of Record: Suzanne A. Sprunger, Ph.D., Reg. No. 41,323

Correspondence Address:
American Home Products Corporation
Patent & Trademark Department - 2B
Attn: Kay E. Brady
One Campus Drive
Parsippany, New Jersey 07054
Tel: (617) 665-8284
Fax: (617) 876-5851

Docket Number: GI 5398

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Drawings, Figures 1-6; 6 sheets

The Commissioner is authorized to charge to required fee of $150.00 to Deposit Account No. 07-1060. Any other fees necessary for filing of the present application may also be charged to such deposit account.

Suzanne A. Sprunger, Ph.D.
Reg. No. 41,323
March 31, 2000

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1 10 on the date indicated above and is addressed to the Assistant Commissioner For Patents, Washington, D.C. 20231

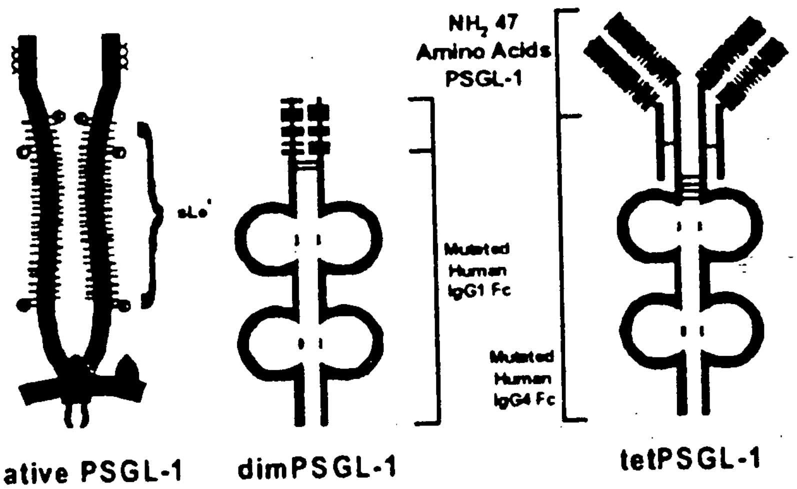


Figure 1. Structure of PSGL-1 Molecules

Figure 2

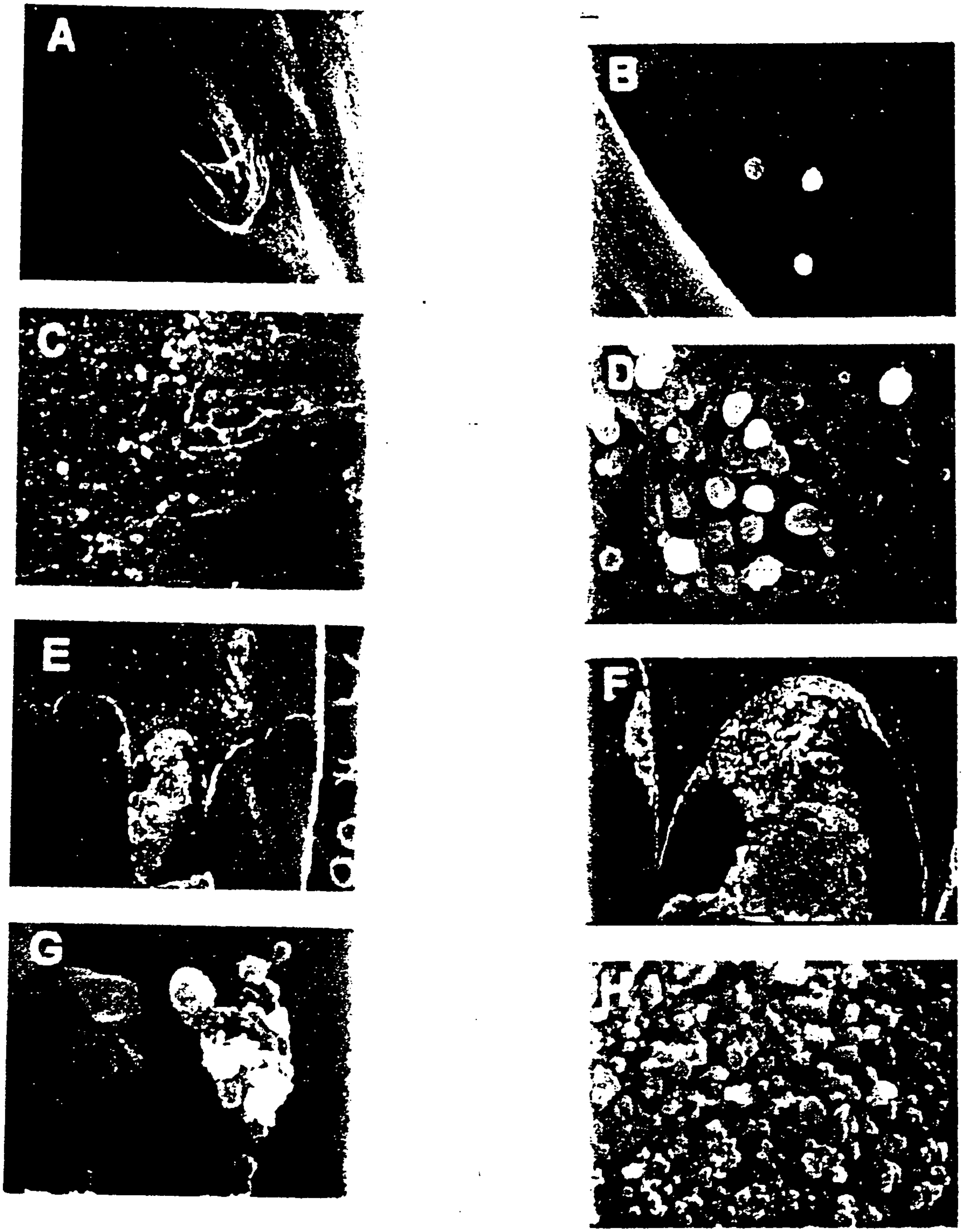
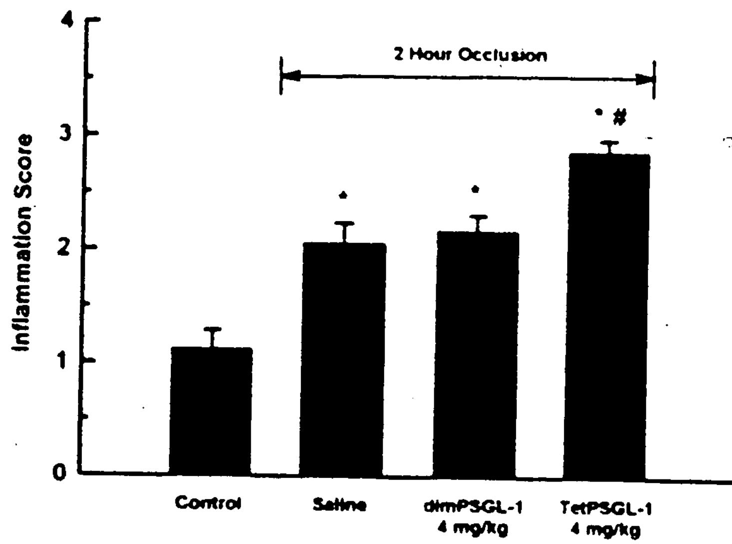


Figure 3



Inflammation Score
0
1
2
3
4
2 Hour Occlusion
Control
Saline
dimPSGL-1
4 mg/kg
TetPSGL-1
4 mg/kg

Figure 4

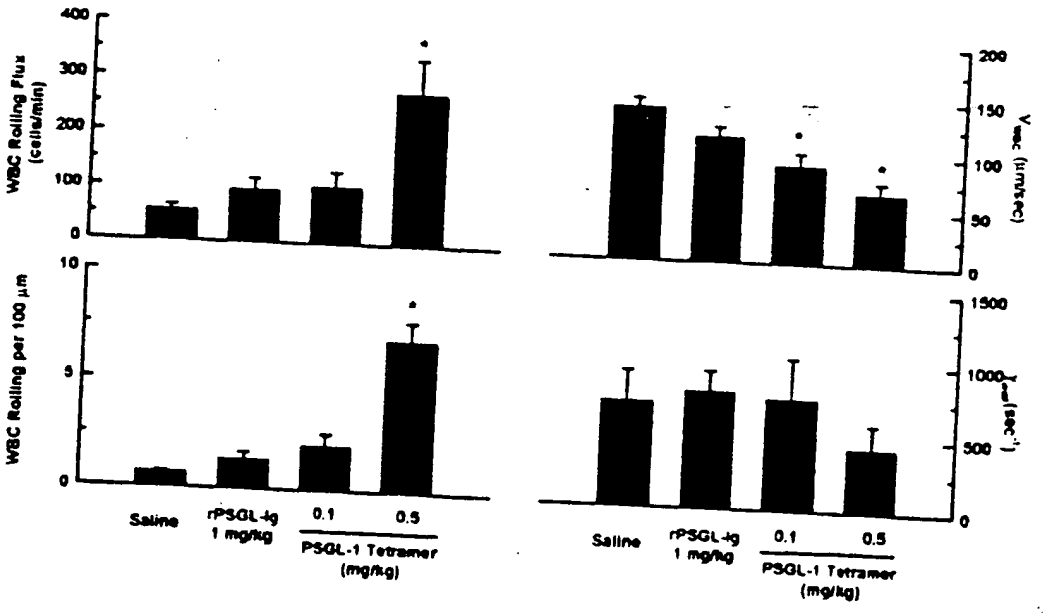


Figure 5

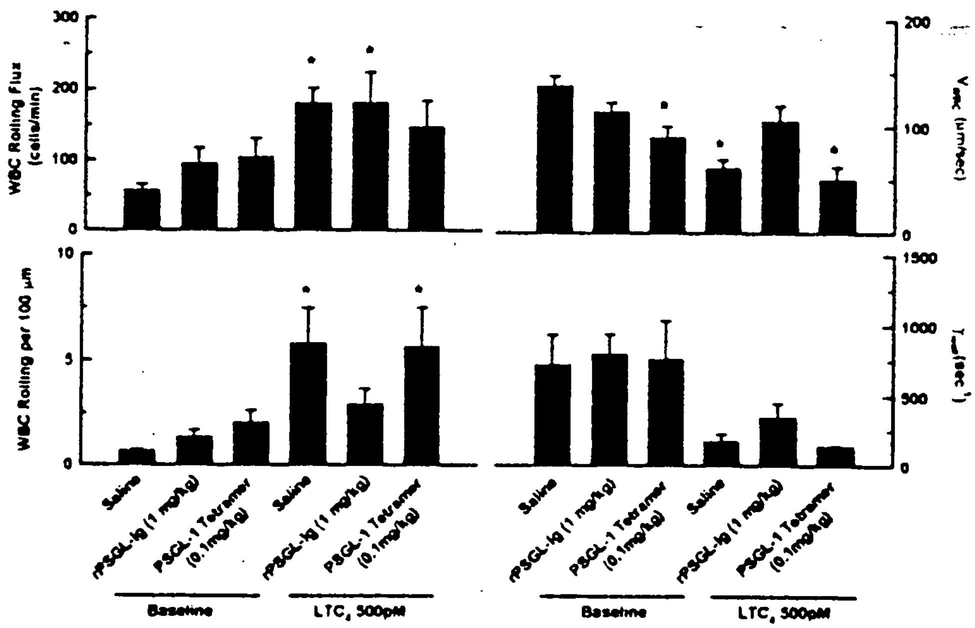
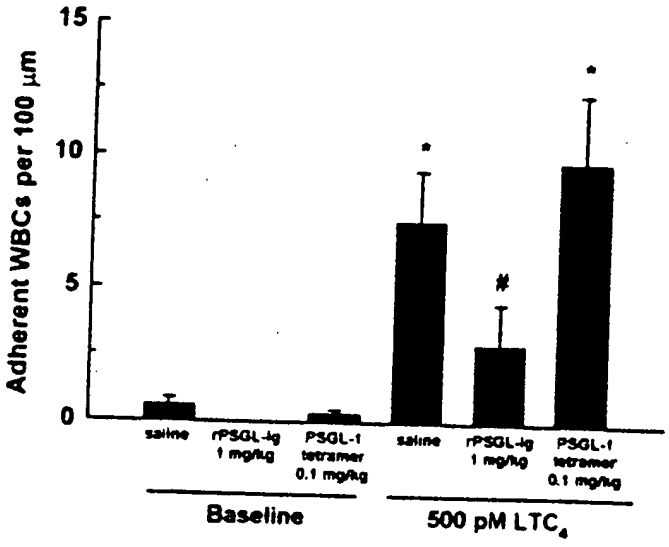

WBC Rolling Flux (cells/min)
WBC Rolling per 100 µm
Saline
rPSGL-Ig (1 mg/kg)
PSGL-1 Tetramer (0.1mg/kg)
Baseline
LTC4 500pM

Figure 6


Adherent WBCs per 100 μm
15
10
5
0
saline
rPSGL-Ig 1 mg/kg
PSGL-1 tetramer 0.1 mg/kg
saline
rPSGL-Ig 1 mg/kg
PSGL-1 tetramer 0.1 mg/kg
Baseline
500 pM LTC4
*
#
*

GI 5398

Inhibition of Thrombosis by Treatment with Dimeric PSGL-1

Summary

- Tetrameric forms of PSGL-1 exacerbate cell adhesion, endothelial cell injury and thrombosis during venous stasis.
- Higher doses of tetrameric PSGL-1 induce a baseline inflammatory response characterized by an elevation in leukocyte rolling and a reduction in rolling velocity.
- Dimeric PSGL-1 molecules inhibit LTC_4-induced reduced in leukocyte rolling velocity and attenuate subsequent adhesion.
- Elevations in avidity of proteins for their ligands do not directly correlate with their ability to inhibit biological functions, compared to molecules with lesser avidity.

Objectives

- To evaluate the effect of dimeric and tetrameric P-selectin antagonists on the rolling behavior of leukocytes in acutely inflamed venules.
- To characterize the effect of P-selectin antagonists to mediate leukocyte and platelet accumulation in a model of deep vein thrombosis (DVT).

dimPSGL-1 was produced by truncating the NH_2 47 amino acids of native PSGL-1, thereby maintaining a high affinity for P-selectin, but dramatically reducing binding to L- and E-selectin (Figure 1) In addition, the NH_2 47 amino acids were linked to a Fc portion of human immunoglobulin-1 (IgG_1). This restored the bivalent presentation observed in the native PSGL-1 molecule. To increase the avidity of a P-selectin antagonists, a tetrameric form of PSGL-1 was constructed by truncating the NH_2 47 amino acids of native PSGL-1 and fusing them to the light and heavy chain regions of the Fc portion of human IgG_4 (Figure 1). Finally, 2 amino acids of the IgG-Fc region have been mutated to disable Fc receptor binding and complement fixation effector functions.

Materials and Methods

Experimental Protocol for DVT

22 male domestic shorthair cats (1.8 kg-3.2kg) were fasted for 18-24 hours and injected intramuscularly (i.m.) with ketamine hydrochloride at a dose of 35 mg/kg. Animals were intubated with a 3-0 gauge endotracheal tube and anesthetize with 1-2% isoflurane at a flow rate of 1 ml/min. Prior to surgery, animals were injected intravenously with either saline (vehicle) (n=7), 4 mg/kg dimPSGL-1 (n=5) or 4 mg/kg tetPSGL-1 (n=5) in 3 ml saline. The necks of the animals were shaved and a incision was made, exposing the underlying jugular vein. The contralateral jugular vein was not manipulated. The jugular vein was gently freed of surrounding connective tissue by blunt dissection. Following exposure, jugular veins were occluded with a vascular clamp for 2 hours. Control veins (n=5) were obtained from animals that were not surgically manipulated, following 2 hours of anesthesia. After venous stasis, side branches and the distal end of the vein were tied off with silk suture and veins were perfused with Ca^{++}-Mg^{+} free Tyrodes buffer to remove non-adherent blood cells,subsequently the vein was reclamped to prevent the re-entry of blood cells. Immediately thereafter, the vein was perfused with 1% gluteraldehyde (in Ca^{++}-Mg^{+} free Tyrodes buffer) and tied off under physiological pressure. Veins were harvested, and prepared for SEM 30-50 regions of a venous segment were observed and given a histological score of inflammation, as follows:

0 - Intact endothelium with no adherent leukocytes and/or platelets

1 - Intact endothelium with some adherent leukocytes and/or platelets

2 -Focal endothelial cell damage with adherent leukocytes and/or platelets

3 - Focal endothelial cell damage with surface thrombosis and/or migrating leukocytes

4-Focal endothelial cell damage with migrating leukocytes, adherent leukocytes and/or platelets and/or fibrin

5 - Extensive endothelial cell damage with migrating leukocytes, adherent leukocytes and/or platelets and/or fibrin

Intravital Microscopy

Male domestic shorthair cats (n=22, body wt=1.8-3.2 kg) were fasted for 18-24 hours before surgery. The animals were initially sedated with ketamine hydrochloride (50 mg/kg, i.m.), and subsequently intubated with a 3-0 gauge endotracheal tube. Animals were anesthetized with 1-2% isoflurane at a flowrate of 1 L/min. The animal's body temperature was maintained at 37 C° by using a heated circulating water blanket. The right carotid artery and left jugular vein were cannulated with polyethylene tubing (PE-190) for the measurement of systemic arterial pressure (Stratham P10EZ and Grass physiological recorder) and the administration of reagents, respectively. Prior to laparotomy, all animals were administered 20 mg/kg sodium cromolyn intravenously to reduce any effects of mast cell degranulation on baseline leukocyte rolling.The animals were placed in a supine position and a midline abdominal incision was made. A segment of the small intestine was gently exteriorized, placed over an optically clear viewing window and superfused with bicarbonate buffered-saline (BBS) (pH=7.4) at 37 C°. All exposed tissues were draped in BBS soaked gauze and covered with saran wrap to prevent evaporation. The mesenteric preparation was observed through an intravital microscope (Zeiss Axioscope FS) with a 40X (NA 0.75) water immersion objective lens and a 10X eye-piece. The image of the post-capillary venule was recorded with a video camera (Panasonic GP-KR222) and a video-cassette recorder (Sony SVT-S3100) for off-line analysis of leukocyte rolling and adhesion.

Single unbranched mesenteric venules (20-45 µm diameter) were selected for each study. Venular diameter (D_{ven}) and red blood cell (RBC) velocity (V_{RBC}) were determined using image shearing and two-slit photometric techniques, respectively. Mean blood velocity (V_{MEAN}) and the Newtonian wall shear rate (γ_{WALL}) were determined as $V_{RBC}/1.6$ and (V_{MEAN}/D_{ven}) x 8, respectively. The number of rolling and adherent leukocytes was determined off-line during playback of videotaped images. Rolling leukocytes were defined as leukocytes that moved at a velocity less than that of RBCs in a given vessel and evaluated using frame-by-frame analysis. The number of rolling leukocytes (flux) were determined by counting all visible cells passing through a perpendicular plane to the vessel axis over a 5 minute period. Leukocyte rolling velocity was assessed by measuring the period of time required for a leukocyte to roll a given distance and averaged for 10 leukocytes per venule. The number of rolling leukocytes present in the venule at any one time was calculated by flux/V_{WBC}, and expressed as number of rolling leukocytes per 100 µm length of venule. A leukocyte was defined as adherent if it remained stationary to the endothelium for longer than 30 sec. The total number of adherent leukocytes was determined over a 5-minute period.

Prior to laparotomy, animals received either 20 mg/kg cromolyn and either saline (control) (n=12), 1 mg/kg dimPSGL-1 (n=5), 0.5 mg/kg tetPSGL-1 (n=3), or 0.1 mg/kg tetPSGL-1 (n=5), intravenously. After exteriorization, the mesentery was allowed to stabilize for 30 minutes. Subsequently, the venule under observation was videotaped for 5 minutes to obtain baseline measurements of leukocyte rolling and adhesion. In addition, baseline measurements of MAP, V_{RBC}, and D_{VEN} were obtained. Immediately thereafter, the mesentery was superfused with BBS containing 500 pM LTC_4 for 30 minutes and the venule was videotaped for 5 minutes to record changes in leukocyte rolling and adhesion. After the 5-minute recording period, measurements of MAP, V_{RBC}, and D_{VEN} were obtained to assess the effect of LTC_4.

RESULTS

Figure 2. SEM micrographs from control cats and cats exposed to 2 hours of venous occlusion in the presence of a tetrameric P-selectin antagonist.

(A) and (B) In control animals, nominal adherence of leukocytes and platelets was observed on venous epithelium and around venous valves. Although some leukocyte adhesion exists, it is evident that the venous epithelium remains intact (61x and 1270x, respectively). Following 2 hours of venous occlusion in untreated cats (C and D), a low magnification view (C) demonstrates a region of adherent leukocytes and platelets. Adhesion of leukocytes and platelets results in the sloughing of endothelial cells (750x). (D) High magnification view of leukocyte emigration and endothelial cell injury, resulting in exposure of the basement cell membrane and platelet adhesion (2700x). Treatment of animals with 4 mg/kg dimPSGL-1 had no effect on leukocyte and platelet adhesion, or endothelial cell injury after 2 hours of occlusion. Panels (E) - (H) are views of jugular veins from animals treated with 4 mg/kg tetPSGL-1. (E) and (F) demonstrate the presence of large thrombi at venous valves (30x and 61x, respectively). (G) Leukocyte-platelet aggregates were observed within and around the thrombus (3660x). (H) Large numbers of leukocytes and platelets were adherent to injured epithelium (1810x).

Figure 3. Effect of dimPSGL-1 and tetPSGL-1 on v nous wall inflammati n following venous occlusion (A) Following 2 hours of occlusion, a significant increase in the level of inflammation was observed, compared to control ($p < 0.05$) and was invariant with administration of dimPSGL-1. In animals treated with tetPSGL-1, inflammation was observed to be exacerbated, compared to control and 2 hours of occlusion ($p < 0.05$). Large thrombi were found in all animals treated with 4 mg/kg tetPSLG-1, compared to an absence of thrombi in saline and dimPSGL-1-treated animals. * denotes value is significantly different from control, $p < 0.05$. # denotes value is significantly different from saline treated animals following 2 hours of occlusion, $p < 0.05$. Values shown are mean ± SE.

Figure 4. Effect of dimPSGL-1 and tetPSGL-1 on leukocyte rolling under basal conditions. (A) Leukocyte rolling flux was approximately 60cells/min in saline treated cats and was invariant with administration of dimPSLG-1 and 0.1 mg/kg tetPSGL-1. Rolling flux was significantly elevated in animals receiving 0.5 mg/kg tetPSGL-1, compared to saline-treated animals ($p < 0.05$). (B) While dimPSGL-1 had no effect on baseline leukocyte rolling velocity, 0.1 and 0.5 mg/kg tetPSGL-1 reduced leukocyte rolling velocity by 35 and 50%,respectively, compared to saline-treated animals ($p < 0.05$). (C) The changes in leukocyte rolling flux and velocity induced by administration of 0.5 mg/kg tetPSGL-1 resulted in a 7-fold increase in the number of rolling leukocytes per 100 μm ($p<0.05$) (D) Wall shear rates in venules of saline, dimPSGL-1, and tetPSGL-1 were not significantly different from each other, suggesting that the changes in rolling behavior of leukocytes is not due to alterations in venular hemodynamics. * denotes value is significantly different from saline, $p < 0.05$. Values shown are mean ± SE.

Figure 5. Effect of dimPSGL-1 and tetPSGL-1 on leukocyt rolling following xposure to LTC_4. (A) Following exposure to LTC_4, leukocyte rolling flux was observed to incr ase 2-3 fold in saline- and dimPSGL-1-treated animals, compared to baseline values ($p<0.05$). (B) LTC_4 reduced leukocyte rolling velocity in saline- and tetPSGL-1-treated animals by approximately 50%, compared to baseline values. However, dimPSGL-1 was effective at abolishing the LTC_4-induced reduction in leukocyte rolling velocity. (C) The changes in leukocyte rolling flux and velocity induced by LTC_4 resulted in an increase in the number of rolling leukocytes per 100 μm to approximately 8 ($p<0.05$). The number of rolling leukocytes per 100 μm was not significantly elevated in dimPSGL-1-treated animals following exposure LTC_4, and was primarily due to the effect of dimPSGL-1 leukocyte rolling velocity. (D) Wall shear rates in venules of saline, dimPSGL-1, and tetPSGL-1 were reduced following exposure to LTC_4, however the values of wall shear rate were not significantly different between treatment groups. * denotes value is significantly different from control, $p < 0.05$. Values shown are mean ± SE.

Figure 6. Effect of dimPSGL-1 and tetPSGL-1 on leukocyte adhesion following exposure to LTC_4. Leukocyte adhesion was observed to be less than 1 WBC/100mm under baseline conditions, and was invariant between treatment groups. Following exposure to LTC_4, leukocyte adhesion significantly increased to 8 leukocytes per 100 mm in saline and tetPSGL-1 treated animals. Administration of dimPSGL-1 reduced LTC_4-induced leukocyte adhesion by 60% ($p<0.05$). * denotes value is significantly different from control, $p < 0.05$. # denotes value is significantly different from LTC_4, $p < 0.05$. Values shown are mean ± SE.

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host

cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunolgobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering

protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on

the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μg to about 100 mg (preferably about 0.1ng to about 10 mg, more preferably about 0.1 μg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically,

systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and

carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorbtion of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

What is claimed is:

1. A method for inhibiting the formation of thrombi by treating a subject with dimeric PSGL-1.

2. The method of claim 1, wherein the dimeric PGSL-1 molecule comprises an immunoglobulin Fc region.

3. The method of claim 1, wherein the subject is human.

4. The method of claim 1, wherein the dimeric PSGL-1 is administered prior to the onset of symptoms associated with thrombus formation in the subject.

5. The method of claim 1, wherein the amount of dimeric PSGL-1 administered is between approximately 0.1 mg/kg and 10 mg/kg.

6. The method of claim 5, wherein the amount of dimeric PSGL-1 administered is approximately 1 mg/kg.

7. The method of claim 5, wherein the amount of dimeric PSGL-1 administered is selected from the group consisting of 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1.0 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 1.75 mg/kg, 2.0 mg/kg, 2.25 mg/kg, 2.5 mg/kg, 3.0 mg/kg, and 3.5 mg/kg.

8. A method for inhibiting cell adhesion to blood vessels by treating a subject with dimeric PSGL-1.

9. The method of claim 8, wherein the potentially adherent cells are selected from the group consisting of leukocytes and platelets.

10. A method for increasing the movement of cells relative to blood vessels by treating a subject with dimeric PSGL-1.

11. The method of claim , wherein the moving cells are selected from the group consisting of leukocytes and platelets.

12. A method for inhibiting the effect of thrombus-inducing agents on cells that have the potential to adhere to blood vessels.

13. The method of claim 12, wherein the potentially adherent cells are selected from the group consisting of leukocytes and platelets

14. The method of claim 12 wherein the thrombus-inducing agent is LTC_4.

Abstract

The selectin (E, P- and L-) family of cell adhesion molecules mediates initial contact between the leukocyte (WBC) and endothelial cell (EC) during inflammation. To inhibit these early adhesive interactions, we have developed several recombinant soluble forms of P-selectin Glycoprotein Ligand-1 (PSGL-1). Dimeric (dim) and tetrameric (tet) forms of PSGL-1 were evaluated using intravital and scanning electron microscopy (SEM) in feline veins. With respect to their ability to bind immobilized P-selectin, tetPSGL-1 was observed to have a 5-10-fold greater affinity compared to a dimPSGL-1. In feline mesenteric venules, administration of 1 mg/kg dimPSGL-1 had no effect on baseline WBC rolling flux and velocity ($p > 0.05$). However, a 0.5 mg/kg tetPSGL-1 was observed to increase WBC rolling flux and decrease WBC rolling velocity by 2-3 fold, compared to control ($p < 0.05$). Following EC activation with leukotriene C_4, PSGL-1 dimPSGL-1 but not tetPSGL-1 was found to attenuate WBC rolling and adhesion, compared to saline. In a model of deep vein thrombosis, dim- and tetPSGL-1 were administered at a dose of 4.0 mg/kg, prior to surgery. Following 2 hours of occlusion, jugular veins were perfused with buffer, fixed, and prepared for SEM. In cats receiving saline, 2 hours of occlusion produced moderate levels of WBC and platelet adhesion, EC injury and no thrombosis. Treatment of cats with dimPSGL-1 had no apparent effect on the magnitude of cell adhesion and EC injury, compared to saline treated cats. However, cats treated with a tetPSGL-1 had an exacerbated level of cell accumulation and EC injury, compared to saline. Furthermore, veins treated with tetPSGL-1 exhibited the formation of large thrombi, which were not present in saline treated animals. These data suggest that protein structure and ligand binding properties of therapeutic agents may contribute significantly to mediating biological responses under basal and inflammatory conditions.